

Green politics make more trouble

The widespread conversion of democratic governments to environmental causes is understandable: it keeps, or even catches, votes. But the consequences are increased costs and the tolerance of irrationality.

BRITISH dock-workers, smarting from the failure in the past month of their strike in defence of lifelong (and, often, hereditary) tenure of their jobs, have improbably in the past week struck two memorable blows in defence of environmental causes. A Soviet tramp steamer was refused permission to land the part of its cargo consisting of PCBs (polychlorinated biphenyls) at Tilbury, in the Thames estuary, while a second carrying a larger quantity of the same material was diverted from Liverpool to the Netherlands. Both cargoes originated in Canada. They were intended for destruction in British incineration plants specially and prudently designed for such purposes. (By operating at high temperatures, they avoid the formation of dioxins.) Although the British dockers have probably by their intransigence increased the risks of general exposure to these supposedly toxic chemicals, they (and the environmental organization Greenpeace, which orchestrated the affair at Tilbury) have become the heroes of the hour.

The truth about PCBs, of course, is that, far from being chemically active compounds that might be capable of acute toxicity, they are chemically inert. That accounts for their industrial use as additives that change the physical properties of materials as different as plastics and transformer oils. The environmental significance of PCBs is that they were, in the 1960s, among the first materials recognized to be ubiquitously distributed in the natural environment, in marine life for example. Like the chemically comparable insecticides such as DDT and, conspicuously, its metabolite DDE, they tend to concentrate in fatty tissues. As with DDT and a host of other widely used chemicals, there are animal feeding tests and *in vitro* proxy tests (such as the Ames test) suggesting that PCBs may be carcinogens, but, of necessity, there is no proof. One of the obvious difficulties is that the commercial materials are mixtures of a hundred or so isomers, of which only a score or so are persistent.

So what exactly is the point that the dockers at Tilbury and Liverpool have established? First, in the wake of a futile strike, the dockers have shown that they still have muscle left. That could easily have been the most important consideration in the dramas of the past week. But environmentalists should not now rely on Britain's dockers to fight other battles for them; automobiles using leaded petrol (gasoline) and fruit grown with the benefit of pesticides and fungicides are unlikely now to be turned away

from British ports, for example. Too many dockers probably regard those commodities as among the essentials of modern life. PCBs, by contrast, being synthetic chemicals whose usefulness is not apparent, are convenient whipping-boys that will already have enabled British dockers to win friends and allies among the great but unfamiliar company of the environmentally alert.

The pity is that the exclusion of PCBs is an assault on the rationality of public administration. If PCBs were dangerous, it would surely be better that they should be safely incinerated than allowed to remain intact. Since nobody has claimed that the materials delivered to Tilbury and Liverpool this past week would have endangered those handling their containers, forwarding them to the intended incineration plants would have been the safest course of action. If there are doubts about the safe operation of the incineration plants, there are also means by which safe operation can be monitored.

None of this implies that there is nothing that needs to be done about PCBs. On the contrary, it is evidently a matter of great interest, and potential importance, that these materials are now so widely distributed in the tissues of seabirds and marine animals. It could well be that, as a consequence, the capacity of marine animals to handle other environmental insults, such as oil-spills, may be impaired. But these are matters for research in a field so complicated that serious work has hardly begun, not for unilateral action by dockers and their provokers. The end result of last week's dramatic pantomime is not merely that irrationality has been lent respectability but that Britain, which is much in need of a more flourishing export business, has been further impoverished.

Much the same is true of the British government's present predicament about water quality. For close on 20 years, the British have been at odds with the rest of the European Communities (EC) on the permissible amounts of nitrate in drinking water. The British safety limit is 80 mg per litre, but the EC has settled for 50. The Royal Commission on Environmental Pollution in Britain considered the issue in 1980, concluding that each limit would ensure that people are unlikely to come to harm. The chief cause for concern about nitrate is that it may be converted by intestinal bacteria into nitrite, which is a known carcinogen. The ratio of the British and EC safety limits is much less than the relative uncertainty of different estimates of how dangerous nitrate in drinking water may



be, which literally means that each of the two limits is equally valid. Yet much of the British public water supply, especially where nitrate fertilizers are intensively used in agriculture, contravenes the EC limit. The consequence is that water utilities will be required to spend extra on treatment plants in the years ahead, while the government has already agreed to compensate some farmers for not using as much fertilizer as they might otherwise have done. That is another pointless but expensive fuss.

Less than a year has passed since the British prime minister, Mrs Margaret Thatcher, took her electors and political opponents by surprise with her declaration that people should worry about the prospect that the accumulation of infrared absorbing gases in the atmosphere may induce climatic change. On that score, Mrs Thatcher is entirely right. But there is also a strong presumption that she, like politicians elsewhere, is seeking not only to take the lead in warning of a substantial environmental danger, but also to win general support from voters inclined towards environmental causes. Certainly there has been little attempt by the British government (or, for that matter, its counterparts elsewhere) to argue that the potential seriousness of the greenhouse effect may limit the attention it is possible to devote to other environmental nuisances. But that is the reality. It is a simple matter of economics. Even if, in the next few years, it is possible to reach an international understanding to limit the quantities of greenhouse gases reaching the atmosphere, the difficulties will only then have begun. Industrialized countries will find themselves having to behave as if the price of energy were much greater than it needs to be. Developing countries, meanwhile, will be denied the more obvious paths to economic development. That is why it gives such great offence that an alliance of British dockers and environmentalists should have chosen to make a pantomimic empty gesture on behalf of PCBs. □

Planck too constant

West Germany's Max Planck Society wrings its hands about immobility in research, but could do much to help.

It is not for nothing that Professor Heinz Staab, the retiring president of the Max-Planck Gesellschaft (MPG), has been ruminating about the future shape of the organization of which he has been the head since 1984 (see *Nature* 340, 335; 1989). The truth is that MPG has become the prisoner of its own success. The network of MPG institutes offers people opportunities to follow careers in basic research untrammelled by the customary academic responsibilities for teaching, let alone administration. Although the MPG network has been at the cutting-edge of the remarkable resurgence of basic research in West Germany during the past 15 years, it is not surprising that many of its institutes have also silted up with people whose liveliness has proved less durable than their tenure of their jobs. That is a familiar problem, but

one that is bound to seem the more alarming in a system in which federal support for basic research is channelled almost equally through MPG institutes on the one hand and through research grants (by the Deutsche Forschungsgemeinschaft) on the other.

Staab's problem will not be solved within the MPG system itself, but only in the broader context of the West German university system. The plain truth is that the university system offers too few opportunities for people in mid-career to become independent scientists with the stature of those called principal investigators in the United States. The need for a means of keeping mid-career people in the university system was recognized, more than a decade ago, by the creation of the system of Heisenberg Fellowships, essentially postdoctoral appointments conceived of as a means by which able researchers could remain in an academic orbit until permanent positions became vacant. The system has been a limited success. Applicants for the fellowships have been of high quality, but the expectations of those appointed have too often been frustrated by the dearth of permanent positions in the university system, which is in turn a measure of the disaffection of *Länder* governments from further university expansion.

The essential stumbling block is the general assumption in West Germany that only those with permanent positions are able to regard themselves as true academics. The old traditions of the *Lehrstuhl*, in which a newly appointed head of a university department might expect to make a deal on equipment and on his annual budget directly with officials of the *Land* government, plainly die hard. But MPG, which has substantial funds at its disposal and also a long-term interest in the encouragement of mobility within the research profession, could do a great deal to help, perhaps by the gesture of offering small and non-tenured research groups in universities an appropriately informal sense of membership in a larger institute, perhaps on a different site. West Germany is not, after all, so huge and uncommunicative that distance would be a difficulty.

MPG itself would benefit from any arrangement that would allow it more convincingly to reject the complaint that its own talent is too little concerned with the education of the young. In reality, of course, many MPG institutes provide important facilities for the education of graduate students. For instance the radioastronomy institute at Effelsburg, near Bonn, is an almost indispensable means by which graduate students earn degrees in radioastronomy. The mathematics institute, also at Bonn, is both a national centre of excellence and almost a part of the University of Bonn. But there are other institutes whose direct contribution to teaching and training in research should be greater and more direct. And there is something in the opinion that the supervision of graduate students is the lesser part of the process of higher education. MPG could benefit not only West Germany but itself by planning more deliberately to integrate its institutes with universities. □

Plant researchers eager for genome programme

- \$500 million US project proposed
- Agricultural competitiveness seen at issue

Washington and Tokyo

Now that the US programme to coordinate the mapping and sequencing of the human genome is up and running, plant researchers are building momentum to launch a programme of their own. At the end of this month, the US Department of Agriculture (USDA) will hold a meeting to sketch out a proposal for a \$500 million project to study plant genomes, steered by the agency's newly created plant genome office. Enhancing US economic 'competitiveness' will be one of the arguments for spending such large sums, but international plant genome efforts are still fragmentary, and it is not clear with whom the United States is meant to compete.

The idea for a USDA plant genome programme was championed by Orville Bentley, who recently retired as head of USDA's research branch. Bentley's successor, Charles Hess, a plant physiologist from University of California-Davis, is likely to support increased funds for plant research, an area that has been chronically poor. The programme will benefit from a call for the USDA to spend ten times more on competitive research grants — or roughly \$500 million per year — included in a report to be released next month by the US National Academy of Sciences' National Research Council.

Jerry Miksche, head of the USDA plant genome office, hopes to receive \$50 million per year for ten years — roughly the amount the USDA has spent on research outside the agency each year for the past ten years. The exact research agenda will be set by the committee meeting at the end of the month, but Miksche says he "wants to avoid" competition between researchers studying different plants. Rather than putting all of its money into one plant species, USDA will concentrate on mapping agriculturally important genes, such as those conferring drought and disease resistance, in several species at once. Miksche estimates that at first 20 per cent of the money will go into developing databases of genetic information, that may or may not be linked to human genome databases.

Under a smaller project, the US National Science Foundation (NSF) is already considering financing a concerted effort to map and sequence the weed *Arabidopsis*. The agency may spend \$35 million to map the weed, which has a genome a little smaller than an average human chromosome. *Arabidopsis* has less repetitive DNA than most other plants,

and is easier to manipulate genetically.

The theme of 'competitiveness' runs strong through the report prepared by USDA officials at the end of last year on the necessity of setting up a USDA plant genome programme. It points out that Japan spends \$200 million on rice genetics and molecular biology, and that Britain has begun to develop a map of the wheat genome, through a consortium of seed companies.

Japan has a tiny project to sequence the rice genome at its Tsukuba science city, with ¥70 million (\$500,000) from the Ministry of Agriculture, Forestry and Fisheries. The project is highly touted, and many hope it will develop into a major

HIPPARCOS

Bad day for astronomers

Kourou, French Guiana

AFTER a perfect launch in French Guiana on 8 August, the European astronomy satellite Hipparcos ran into trouble when the apogee motor failed three times to respond to commands to fire. The motor is responsible for lifting the satellite into geostationary orbit around the Earth. As *Nature* goes to press, there are still hopes that the motor will eventually fire.

Two more attempts to fire the motor are planned for 15 and 16 August. For the moment, the satellite remains in an elliptical orbit moving between 208 kilometres and 35,000 kilometres from the Earth. As a last resort, the motors used to orient the satellite will be used to push it into a higher orbit. Many of the experiments could still be carried out, but using the fuel for this purpose would leave the satellite with a much shorter working life.

The Ariane rocket left the launch pad

The easy part: the 9 August lift-off from Kourou went well (AP).

initiative, but researchers involved with the project complain that they barely have enough money to work out the technological hurdles that must be overcome. A more significant effort is represented by Plantech Research Institute, a private joint venture of Mitsubishi Chemical Industries Ltd and Mitsubishi Corporation, that has an annual budget of ¥400 million (\$3 million), much of which it spends on rice genetics.

The British consortium — organized by Agricultural Genetics Company Ltd in Cambridge — consists of five companies spending a total of around £1.5 million to produce rough maps of the genomes of wheat and barley. The UK Agriculture and Food Research Council (AFRC) has a three-year £14 million programme on plant molecular biology, part of which will involve mapping and sequencing. AFRC's John Innes Research Institute is working on mapping *Arabidopsis*. But no major initiative has yet been announced by Britain or the European Community.

Carol Ezzell and David Swinbanks

carrying a West German direct broadcasting television satellite, TV-SAT 2, as well as Hipparcos. The first launch attempt was stopped seven seconds short of ignition by an overzealous computer. But then the mission got off to a good start with the perfect positioning of Hipparcos and TV-SAT 2 in their transfer orbits. Just 37 hours later the problems began.

Hipparcos, named after the Greek astronomer who drew up the first star catalogue during the second century BC, is the world's first astronomy mission, designed to measure the precise positions, parallaxes and proper motions of stars. During its two and a half years of life, the satellite is expected to measure the positions of 100,000 stars with a precision of 0.002 arc seconds. The results, as well as those of the Tycho experiment, designed to measure 400,000 stars with an accuracy of 0.03 arc seconds, should be ready by 1995. And the data should be 50 times more accurate than the best available today, according to Michael Perryman of the European Space Agency (ESA).

The satellite is a 23-year-old dream of a French astronomer, Pierre Lacroute, who was one of the guests at the launch last week along with the French minister for research and technology, Hubert Curien. It was built by the French company Matra and the Italian company Aeritalia at a cost of about \$360 million, and is not insured, according to Roger Bonnet, director of ESA's scientific programmes. A spokesman for Matra said that to build a replacement would take more than three years.

Ricardo Bonalume Neto

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Science ahead of the law

Washington

LAST week, a judge in a Tennessee courtroom was asked to decide if seven frozen human embryos were just 'property' and could be disposed of in the same way as other commonly-held goods in a divorce case, or should be considered as 'human life' with rights of their own. In Missouri, another judge is about to consider whether the controversial Missouri act restricting abortions really gives full rights to a fetus. If so, the judge may have to find a way to set free a fetus that a pregnant prison inmate claims is imprisoned illegally.

The Tennessee case involves a couple who are suing for divorce after nine years of unsuccessful attempts to produce a child. Among their 'jointly-owned property' are seven fertilized eggs (pre-embryos), which are in frozen storage. The eggs were removed from the wife and fertilized *in vitro* with the husband's sperm.

The husband argues that he has a right not to become a father. In his divorce petition, he asks that the court prevent his wife from implanting the eggs. His lawyer maintains that the seven embryos have no human characteristics beyond bearing human genetic material. They are less than 14 days from the time of conception, and are simply a small number of undifferentiated cells. The husband asks that the embryos be kept frozen indefinitely.

The lawyer for the wife argues that the embryos are human life and should be treated as 'children'. Custody over them should be granted to her wife and she should be allowed to implant them in order to become pregnant.

Pre-embryos are often frozen as a part of the effort to obtain a pregnancy through *in vitro* fertilization. Many eggs are usually removed from the potential mother's ovaries at one time and, after fertilization, they are allowed to advance to the eight-cell stage before some are selected for implantation. Any 'spare embryos' left over may be frozen so that they can be used again if no pregnancy has occurred.

There are continuing arguments over whether those spare embryos can be donated to women who either cannot produce eggs, or who have genetic deficiencies that make it inadvisable that they should do so. The fiercest disputes are over whether pre-embryos can be discarded or used in research — eleven US states have banned research on human embryos.

The Tennessee case is the first time these issues have come up in a divorce proceedings. The lawyer for the wife opened the case by stressing that the central issue is "where life begins". He asked that the pre-embryos be labelled "pre-born children". Jerome Lejeune, a French

researcher who discovered the cause of Down's syndrome, agreed. He testified that pre-embryos are human life and referred to them as "tiny human beings". But John A. Robertson, a law professor from the University of Texas, argued that although the pre-embryo deserved a special respect as a potential person it should not be granted the same rights as a person.

The Judge is expected to rule on the case in 30 days time. It is the first ruling of biological importance to come out of a Tennessee courtroom since the famous Scopes trial on evolution.

In Missouri, it is the apparent success of the anti-abortion lobby in persuading the state to incorporate into law the declaration that "life begins at conception" and that unborn children have "all the rights, privileges and immunities available to other persons..." that is causing new ethical difficulties. The controversial act survived Supreme Court scrutiny earlier this year and reopened the national battle over the right to an abortion (see *Nature* 340, 83; 13 July 1989).

The new act has permitted an inmate of a Missouri prison to file a lawsuit against the state for illegally imprisoning her fetus. The lawyer for the woman argues that as the act has granted 'personhood' to the fetus, it cannot be sent to prison except as a punishment for a crime.

Alun Anderson

COLD FUSION

Utah backs new centre with \$5 million

Washington

THE state government of Utah has decided to support research into cold fusion, despite growing apathy elsewhere, by spending \$5 million to establish the National Cold Fusion Institute at the University of Utah in Salt Lake City. The money was put aside when news of the claimed discovery by Stanley Pons and Martin Fleischmann first emerged (*Nature* 338, 447; 1989) but although some \$500,000 has been spent on legal fees and the filing of patents, the remainder had been kept back pending scientific confirmation of the reality of the phenomenon.

But last week, confirmation or not, an advisory panel to the state legislature voted by seven to one to release the money. The physicist on the panel, Wilford Hansen of Utah State University, abstained.

The \$4.5 million will be used over the next two years to establish a laboratory in an already existing building on the research park site of the University of Utah. As many as 40 people may be hired, and senior staff of the National Cold Fusion Institute will be faculty members of the University. For the time being, Hugo Rossi, a professor

Moons and rings for Neptune

Washington

VOYAGER 2, closing in on its 25 August rendezvous with Neptune, has spotted three more previously unknown satellites and has confirmed the existence of partial rings, or rings arcs, around the giant planet. The three new moons all measure about 100 km across and, like the first satellite detected by Voyager (*Nature* 340, 86; 1989), have roughly circular equatorial orbits, with radii ranging from 50,000 to 75,000 km.

The existence of partial rings around Neptune had been inferred by astronomers who had seen unexpected occultations of stars passing behind the planet. Voyager's new photographs, received at the Jet Propulsion Laboratory in Pasadena, California, on August 11, show arcs of material apparently associated with two of the newly found moons: one arc lies just outside the innermost moon, 1989 N4, and the other lags behind 1989 N3, the next moon out, by about one quarter of an orbit. The arcs are thought to consist of small particles of debris, 'shepherded' by the gravitational influence of the nearby moons.

David Lindley

■ THE Space Shuttle Columbia landed at Edwards Air Force Base in California last Sunday after successfully completing a five-day secret defence mission. The flight restores the shuttle fleet to its complement of three orbiters. Columbia is the oldest of the shuttles and first flew in 1981. □

of mathematics at the University of Utah, will act as director of the Institute, but a search for a permanent director has begun.

Whether the new Institute will have anything of substance to conduct research on is still an undecided matter. Outside Utah, few other claims of significant heat production from palladium electrodes in heavy water are being heard, and a number of negative results have now been published. (On page 525 of this issue of *Nature*, Nathan Lewis and his colleagues give an account of the failure of a series of experiments at the California Institute of Technology to unearth anything out of the ordinary.)

Nevertheless, the University of Utah has seven cold fusion patents under consideration, and has two more applications almost ready to be submitted. A spokesperson for the university said that they had received no word yet on their applications, and that it might well be two or three years before a decision was made. The backlog at the General Patent Office in Washington is such that ten months can easily elapse before a submitted application is even examined.

David Lindley

Britain drops ESA project

London

THE UK Science and Engineering Research Council (SERC) has pulled out of a multi-million-pound European Space Agency microgravity research programme. The decision was made after a report prepared by Sir Brian Pippard, of the University of Cambridge, estimated the cost of maintaining a "useful presence in microgravity" at £8 million a year.

In a response to the report, *Prospects for British participation in microgravity research*, SERC chairman E.W.J. (Bill) Mitchell concluded that "there is insufficient evidence of scientific promise to justify our involvement in a major programme". Pippard, who considered the



funds available to British scientists, said he could find no "strong case" for microgravity research.

Critics of SERC's decision to forgo the microgravity programme say the selection of Pippard, a physicist and material scientist, prevented an accurate appraisal of

the biological importance of the mission. At least half the microgravity programme is concerned with fundamental research in biology. They say Pippard's claim that "microgravity promises no more than an inward consolidation, tidying up neglected areas of a science that is already so well delineated as to be termed classical" is flawed.

Professor Heinz Wolff, of the Institute for Bioengineering at Brunel University and chairman of the ESA Microgravity Advisory Committee, said the Pippard report misrepresented the scientists who are keen to see the programme go ahead. Wolff, who is working on the JUNO project, which will see British scientists involved in microgravity experiments on the Soviet Mir space station, said that he had already received many microgravity experiment proposals. That "would seem to contradict the assertion that interest in the UK is small", he said.

Professor Kenneth Pounds, of the University of Leicester, also regrets the SERC decision. He said the decision was based on a desire for "short-term returns" on investment in research and added that it was a lost opportunity for research into areas of biology, physics, chemistry and material science. "Other European countries and the Americans believe it is a great chance to carry out basic research. Pippard has thought about the most predictable opportunities and concluded it is not worth it. For even a modest sum we could include Britain in what could develop into a very exciting field."

Ben Webb

ROCKET LAUNCH

First setback for Japan

Tokyo

JAPAN'S National Space Development Agency (NASDA) suffered a setback last week when the agency's H-1 rocket sat on the launch pad unable to lift off after its first-stage main engine had fired. This is the first launchpad failure for Japan and comes on top of problems the agency is having with the development of its next-generation H-2 rocket (see *Nature* **340**, 253; 1989). Delays to Japan's space programme may result.

The H-1 rocket was to have carried a meteorological satellite (GMS-4) into geostationary orbit. But after the main engine fired, a fuel valve on one of the six auxiliary rockets attached to the first stage failed to open and the engine was automatically shut down.

NASDA hopes to re-schedule the launch this year but has only a very limited launch window in which to do so. Under an agreement with fishermen in the area of

NASDA's space centre on Tanegashima island, rockets can be launched only during two 45-day periods each year in summer and winter. The present launch window comes to an end in the middle of next month. The rocket cannot be launched between 17 and 27 August because the tracking facilities of the US National Aeronautics and Space Administration which are required for tracking the H-1 launch will be monitoring the interception of Voyager 2 with Neptune. That leaves only about two weeks for a launch this year. Otherwise the launch must be postponed until early next year.

David Swinbanks

■ Japan's efforts beneath the ocean met with better fortune last week, when the submersible *Shinkai 6500* achieved a world record depth of 6,527m in the Japan Trench. The previous record was 6,170m set by the Soviet vessel *Mir* in 1987.

Royal Society suspends collaborations

London

THE Royal Society of London has announced that it is to suspend some of its collaborative agreements with Chinese government bodies.

The society says that because of the "tragic events" in China it will cut relations with the Ministry of Geology and Mineral Resources and has suspended its memorandum of understanding with the National Science Foundation of China. In a statement, the society explained: "We do not intend to make grants to British scientists to attend international scientific meetings held in China until it can be sure that such

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A member of the Chinese Solidarity Campaign beats a traditional drum during the unveiling of a replica statue of the 'Goddess of Democracy', across the road from the Chinese Embassy in London's Portland Place.

meetings are fully international and that attendance by British scientists will not be used to imply support for the government policies".

The society will continue to help individual British and overseas scientists to "develop and maintain contact for their mutual benefit — irrespective of political considerations". The society says it will maintain its support for visits arranged by the Chinese Academy of Science, the Chinese Academy of Medical Science and the China Association for Science and Technology.

Ben Webb

Objections to Japan's system

Tokyo

SUCCESSFUL applicants for big grants are usually the last people to complain of unfairness in the grant selection process. But it seems the Japanese are different: this year, more than a few of the just-announced winners in the competition for Japan's most lucrative basic research grants are ready with criticism of the way they were selected. And they find their opinions echoed by the handful of foreigners who have just encountered Japanese peer review under the Human Frontier Science Program.

Much of the criticism is directed at the large 'special distinguished' and 'priority' grants of the Ministry of Education, Science and Culture (see below). These million-dollar grants run for three to five years. Special distinguished grants are awarded to about ten individuals each year and are intended to support "internationally recognized research that is likely to produce outstanding results". The priority grants support research by large teams of scientists and about 20 are awarded each year.

The grant applications are screened by small committees of academics selected by the ministry's science council, a group of prominent academics and industrialists. After initial screening, potential candidates are summoned to the ministry for interview by one of three committees specializing in biology, literature, and physics, chemistry and engineering. The interview lasts about 20 minutes with a 10 to 15-minute presentation by the applicant followed by questions.

One recipient of a special distinguished grant said he was "deeply depressed" when he entered the room for his interview and discovered that there was no-one among the ten committee members with expertise in his field of research. He said it was clear from their questions that the committee members did not really understand what he wanted to do. He was also dismayed that the committee chairmen wielded so much control over the proceedings and questioning.

The recipient suspects his application was successful only because he happened to have met two of the committee members previously, and because he was able to produce an article praising his work that had been published in a foreign journal. But in the absence of such evidence of international recognition, he says it is easy to see how the committee could make mistakes.

A member of the ministry's science council defends its methods, saying that a researcher with a good proposal should be able to present its aims clearly to intelligent non-specialists. And in his opinion, while the committees may have dropped

good proposals they have never made mistakes in those selected.

But some members of the ministry's grant selection committees think the process could be improved. One solution that has received support is to widen the scope of opinion by involving some foreign experts in the selection of grants.

Another recipient of a priority grant described the selection process as "feudalistic", with a few prominent academics scattering grants among people with a wide range of ability from "aristocrats to peasants". He further complains that many of the recipients are people nearing retirement who seem to have been given awards as a sort of "golden handshake". But he says the grant system has improved in recent years. In the past, recipients of these big grants were chosen by prominent academics without competition. But now anyone can apply. This, however, has created another problem.

Only about one in ten applicants

succeed and those who fail receive no explanation of why their application was rejected. This is in contrast to the grant systems in the United States, for example, where grant applications are sent to expert referees for comment.

The same criticism has been made by Western scientists of Japan's new Human Frontier Science Program. Last year, the Ministry of International Trade and Industry (MITI) launched a 'pilot' Frontiers programme and solicited applications for five international grants from scientists of the Western summit nations and European Communities. But MITI received more than 40 applications and did not explain to the many unsuccessful applicants why their proposals were rejected. According to Japanese scientists involved in the project, several Western scientists were "furious" about the lack of explanation.

A Japanese academic adviser for Frontiers says the system will be revised to accommodate these complaints when the full-scale programme is launched at the end of this month. **David Swinbanks**

More money for cancer

JAPAN'S biggest grants for basic research this year go to the study of protein kinase C and cancer. Research on the global environment, neurocomputing and stellar formation also feature prominently in this year's 'special distinguished' and 'priority' grants announced by Japan's Ministry of Education, Science and Culture.

The special distinguished grants just announced, which go to individuals, begin immediately. But the priority grants which support large teams of scientists do not begin until next fiscal year and thus their size is still subject to negotiations with the Ministry of Finance. Nevertheless, the amount of funds that the researchers applied for gives a good indication of final grant size.

Yasutomi Nishizuka of Kobe University School of Medicine gets the biggest special distinguished grant with an award of ¥330 million (\$2.4 million) over four years to continue his internationally recognized research on protein kinase C. Kumao Toyoshima seems set to get the biggest priority grant with his request for ¥2,400 million (\$18 million) over four years for work on the mechanism of cancer, including oncogenes and viruses. Toyoshima will head a huge team of about 100 researchers divided into 35 or 36 groups. Shunichi Amari, a mathematician at Tokyo University who has developed key parts of the theory behind neural networks models, is also listed for a large priority grant. His team has requested ¥1,433 million (\$10 million) over five years to develop a theory of information-processing in the brain with the ultimate

aim of building a neurocomputer.

Osamu Nishikawa of Risho University's department of geography has requested ¥694 million over three years to develop a computer system to model the effects of Japan's modernization on the global environment. And Kei Takeuchi of Tokyo University's Research Center for Advanced Science and Technology requests ¥799 million for five years to try to dream up socio-economic systems for the twenty-first century that will allow economic growth for developing countries and yet at the same time will protect the environment and natural resources through the utilization of 'environment-friendly' leading-edge technology.

One of the biggest special distinguished grants (¥307 million for four years) goes to Yasuo Fukui of Nagoya University who will use a new 4-metre radio telescope to observe the detailed carbon-monoxide spectra of interstellar gas during the process of star formation.

In addition to awards for internationally recognized research, the ministry has awarded special distinguished grants for research well out of the mainstream. Taizo Masumi of Tokyo University gets ¥144 million to pursue his belief that there is a relationship between photoconductivity and superconductivity in the new high-temperature superconductor materials. And Akimasa Masuda of the same university is awarded ¥214 million to search for two as yet undiscovered isotopes of technetium, a radioactive element with an atomic number of 43.

David Swinbanks

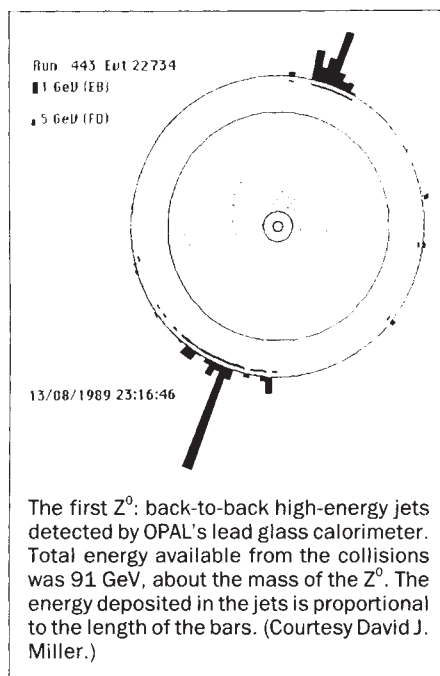
PARTICLE PHYSICS

CERN's LEP makes its first zeds

London

THE new Large Electron-Positron collider (LEP) at CERN, the European Laboratory for Particle Physics, has produced its first Z^0 particles only four weeks after being brought into operation. The first one was spotted by the OPAL collaboration at midnight on Sunday 13 August, just 16 minutes into the first experimental run on the machine. Another four were seen during the next two hours.

The Z^0 was first observed in collisions between protons and antiprotons at CERN in 1983, earning the Nobel Prize for Simon van der Meer and Carlo Rubbia, now CERN's director. By producing and studying Z^0 s in quantity, physicists should be able to resolve many of the uncertainties in the standard model of the fundamental forces. The Stanford Linear Collider (SLC) in California, an upgraded version of an earlier electron accelerator, has been making Z^0 s since April this year, but LEP is expected to outperform SLC significantly. The 27-km circular machine should produce about a million Z^0 s a year, whereas SLC, although it is still not running fully to



design specification, has made 240 in its first four months of operation.

The detection rate at LEP should soon increase by a factor of at least 400 because the beams are now running at only one-tenth of their design current and because the quadrupole focusing magnets, which squeeze the beams at the collision point, are not yet in use. David J. Miller, a member of the OPAL collaboration, says that the four weeks taken from passing the first beams to seeing the first Z^0 s is a remarkably short time in accelerator physics — possibly a record.

Roland Pease

NAGYMAROS DAM

Controversy persists

London

LASZLO Udvari, Hungary's commissioner for the controversial Gabčíkovo-Nagymaros hydroelectric project, last week denied Austrian press reports that construction work at Nagymaros will resume in November. The Austrians have a big financial investment in the Hungarian end of the project, which, if the Nagymaros dam is completed, will be repaid in electricity. Last May, following several years' campaigning by environmental groups in Hungary, the Hungarian government announced a two-month moratorium on construction at Nagymaros. This moratorium expired in July, but work at Nagymaros is still continuing only at the minimum level needed to maintain the temporary coffer-dam around the uncompleted structures. Now, an expert commission from the Hungarian Academy of Sciences is advocating a moratorium of several years to allow a "realistic" assessment to be made of the environmental aspects of the problem.

Previous Hungarian official reports have concluded that although the scheme was a planning error, it would now be too costly, both financially and politically, to cancel, and that with sufficient additional investment the environmental damage could be minimized. The latest academy report, however, says that previous surveys have failed to pay sufficient attention to the protection of water quality, although 3 million Hungarians (out of a population of 10.5 million) drink the filtered water of the Danube.

But cancelling the Nagymaros dam would not save Hungary's water supply, although it would reduce the damage. The main problem, the Hungarian experts say, will be the Gabčíkovo dam, upstream in Czechoslovakia. And, although individual experts from the Slovak Academy of Sciences are now beginning to voice their doubts, the Prague government remains firmly committed to its side of the joint project. Not only does the Czechoslovak economy need the electricity that Gabčíkovo will produce, but the scheme, originally hailed as a triumph of Socialist cooperation, continues to appeal to a regime committed to the principles of the Brezhnev era, including its sponsorship of grandiose 'prestige' engineering projects.

The Czechoslovak government is therefore doing all it can to persuade the Hungarians to complete their side of the deal. At a bilateral conference on the dams in Budapest last month, the Czechoslovak team refused to take seriously Hungarian arguments that the Nagymaros dam lies on a tectonic fault, with the consequent risk from earthquakes. Czechoslovak experts, said Dr Ludvik Vanel, a corres-

ponding member of the Czechoslovak Academy of Sciences, consider that the tectonic disturbances recorded at the site are "quite common" and that all that is needed is a "network of technical stations" monitoring the two dams. The Czechoslovak team particularly objected to the presence in the Hungarian delegation of representatives of 'alternative' environmental groups, who, they claimed, were "inadequately informed". Yet, said Hungarian Academician Pal Stfanovits, the major objections to the dam are based on work carried out by the Hungarian National Geological Institute over almost 10 years, which was ignored by both the Czechoslovak and the Hungarian designers.

The Budapest conference resolved nothing. The Prague politicians and the Austrian investors continue to scold the Hungarians on treaty and contractual obligations and to press their claims for compensation. To this, Commissioner Udvari has replied that if the ecological and natural risks apply not only to the Hungarians but also to the inhabitants of Czechoslovakia and Austria (as in the case of seismic risk), then there can be "no justification" for compensation claims.

But even if the Hungarians do get the "several years" moratorium they are now seeking, further construction work will be needed at Nagymaros in the meantime. For, according to experts, the temporary coffer-dam around the site will not endure the rigours of more than two or three Danube winters.

Vera Rich

ATMOSPHERIC SCIENCE

New German programme

Munich

ENCOURAGED by a parliamentary Enquete commission established last year "for the protection of the earth's atmosphere", Research Minister Heinz Riesenhuber last week announced an initiative consolidating West German efforts in research into atmospheric science and climate change into a new "priority programme". West Germany will invest annually, beginning in 1990, DM 10–15 million of new money into projects meant to provide more data for politicians who want to protect the environment.

In addition to supporting the "Climate Computing Centre" in Hamburg, which bought a Cray-2 supercomputer last October, the new programme is expected to focus on the roll of the ocean as a possible sink for man-made carbon dioxide. The role played by steam, clouds and aerosols in global warming will also be studied.

Steven Dickman

All right for some

Washington

NEXT month, when the US Congress returns from the summer recess, it will have few hard decisions left to make on the 1990 science budget. Although the Senate is way behind the House of Representatives in voting on the key items, the shape of the budget is already clear from discussions at the subcommittee level.

The National Aeronautics and Space Administration (NASA) is to be specially favoured, with a 14.8 per cent increase voted by the House of Representatives, and the National Institutes of Health (NIH) with a 10 per cent increase. There is good news for the space station, which emerged from a budgetary assault with only minor damage, and the Superconducting Super Collider, with its first construction funds agreed. But the National Science Foundation (NSF) will probably have to postpone its long-term plans for a budget doubling yet again.

Health and Human Services. The House of Representatives has given the NIH its usual favourable treatment with an appropriation of \$7,678 million, an increase of almost \$150 million from the president's request, and an increase of more than 10 per cent on the 1989 budget.

The main increases are directed towards research grants (\$44 million), buildings (\$60 million) and high-priority research projects (\$32 million). In order to allay fears that money is being drained from other areas, AIDS research was not treated as a separate item.

Support for the National Center for Human Genome Research appeared as a separate item for the first time, but the House trimmed the administration's request for \$100 million to \$62 million, still more than double the 1989 funds.

Senate has not yet voted, but is expected to agree to the House provisions.

NSF. The House of Representatives dealt the NSF an unexpectedly severe blow, cutting the administration's request by \$150 million to \$1,999 million, only \$7 million more than the 1989 budget. But about half of the reduction was made by transferring half of NSF's budget for the Antarctic programme to the Department of Defense.

Unless NSF is rescued by the Senate, which has yet to vote, expectations of a 14 per cent increase in the research budget will not be met. Although the House agreed to \$1,715 million, an increase of 8.3 per cent on the 1989 research budget, it is still \$88 million less than NSF's request, and further cuts are expected in final budget adjustments. The House recommended cuts across the board rather than in specific programmes.

Congressional concern about standards of science education in schools is reflected

in the appropriation of \$240 million for NSF's educational activities, an increase of 22 per cent above the 1989 budget.

Department of Energy. The budget contains the first funds for construction of the Superconducting Super Collider (SSC). But a compromise has yet to be reached between the Senate's allocation of \$225 million and the House's \$220 million, both short of the \$250 million request.

Cuts made by the administration were replaced with increases for research into renewable energy sources.

The House appropriated \$317 million for the department's biological and environmental research, \$50 million more than the request, but the final amount may be smaller after negotiations with the Senate which appropriated only \$277 million, a 6 per cent increase on 1989. Both allocated about \$50 million for research on the 'greenhouse effect'.

NASA. Frustrated by overall budget constraints, the House cut more than \$500 million from the administration's \$5,751 million request for NASA in 1990. Almost \$400 million of the reduction was made in the \$2,050-million request for the space station. Although large savings can be made by reducing overheads, the cuts will

JAPANESE INVESTMENT

New centre for Massachusetts

Boston

HARVARD-affiliated Massachusetts General Hospital (MGH) announced last week that it is to receive \$85 million over the coming decade in the largest corporate grant ever made to a hospital in the United States. The money comes from Shiseido, Japan's biggest cosmetic company, and is to be used to set up a new dermatology research centre. The new MGH-Harvard Cutaneous Biology Research Center will employ about 100 scientists and support staff. Research at the centre will include the effects of ultraviolet radiation on the skin.

Under the new agreement between MGH and Shiseido, MGH will have the opportunity to patent inventions, but Shiseido will have the right of first refusal to license any such patented research.

The new agreement furthers a trend towards increased corporate funding of research at MGH. Martin Bander, a spokesman for the hospital, says that industry now provides 18 per cent of the hospital's research budget, up from 4.5 per cent a decade ago. Commenting on the fact that the largest deals are with foreign companies, Bander said it was "unfortunate" that more US companies are not willing to support basic research.

Seth Shulman

inevitably delay the programme. NASA's total budget will, however, still be almost 25 per cent greater than it was last year. The House approved the request to increase the life sciences budget by almost 60 per cent to \$124 million.

And although there was a small decrease in funds for planetary exploration, the \$30 million request for the CRAF-Cassini programme was met.

Other agencies. Congress will negotiate in September over the 1990 budget for the Strategic Defense Initiative, but as the House agreed to only \$3,100 million it is certain that the administration's request of \$4,600 million will not be met.

The White House Office of Science and Technology Policy is still on course for a 28 per cent increase in its budget for 1990. The House appropriated slightly more than \$2 million, an increase of almost \$500,000 from 1989, as the administration requested.

The House reinstated funds cut from the budget of the Environmental Protection Agency in the administration's 1990 spending plans, leaving a budget of \$5,422 million, a 5 per cent increase from 1989.

The bill included \$1.5 million and 15 staff for the agency to undertake long-term environmental monitoring in areas with major oil spills, including Prince William Sound, Alaska.

Christine McGourty

NUCLEAR POWER

Glasnost comes to French reactors

Paris

NEW measures have been announced by the French ministries of health, of industry and of the environment to improve the quality of information available to the public about radiation levels around nuclear power plants and waste-treatment sites. The moves follow a promise by Prime Minister Michel Rocard in April to make all information about radiation levels in the environment accessible.

Local radiation levels in samples of water, air, milk and vegetables will be published in the press, at the sites themselves and at prefectures (community administration centres). The same information will be available nationally through the Minitel network of teletext receivers. Bulletins will be published monthly using measures taken by the organizations running each site, such as the atomic energy commission (CEA) and the French electricity utility (EDF). In addition, monthly statistics on ambient air radiation taken at six centres by the institute for the protection of nuclear safety (IPSN) will given in the MAGNOC Minitel bulletin.

The health minister, Claude Evin, has set up an interministerial commission to establish norms for levels of radiation in the environment and in foods. **Peter Coles**

BIOTECHNOLOGY REGULATION

North Carolina adopts its own rules

Washington

IN a move that could usher in a state-by-state approach to the regulation of biotechnology in the United States, the state of North Carolina voted last week to adopt its own rules governing the release of genetically engineered organisms into the environment. North Carolina's legislature passed a law requiring state permits for all field tests of genetically engineered organisms, in addition to the approval already required by US government agencies.

The law — drafted by an unlikely coalition of environmentalists, academics, state government officials and biotechnology industry representatives — seeks to protect the public and prevent local opposition to field tests. All the field tests of genetically engineered organisms carried out so far in the United States have met with some resistance at county or municipal level — with the 1986 California test of the 'ice-minus' bacteria that would shield plants from frost as the most notorious example. By setting up an in-state review system, North Carolina aims to reassure its citizens while encouraging biotechnological innovations that could benefit its large agricultural industry.

The North Carolina law creates a 10-member Genetic Engineering Review Board that includes the state's commissioner of agriculture, a working farmer, someone from a 'public interest' organization and a representative from the biotechnology industry. The board will write detailed regulations that the state's agriculture department will use when it begins to evaluate field test requests next year. The rules will apply to both academic and industrial researchers. Those who conduct tests without approval will be fined; there is no limit on the amount of damages an infringer would pay if an experiment were to go awry.

The US Environmental Protection Agency (EPA) has been struggling for more than two years to amend the Toxic Substances Control Act to cover biotechnology (see *Nature* 337, 681; 1989). One proposal, vigorously opposed by biotechnology companies, would have created local boards to review proposed experiments. EPA officials have expressed puzzlement over why the biotechnology industry should now embrace similar regulations.

Other states may follow North Carolina's example. Illinois and Wisconsin already require notification of field tests of genetically engineered organisms, and in June this year Minnesota set up an Environmental Quality Board with the aim of coordinating state and federal regulations pertaining to such tests. The states of New York, New Jersey and Washington are also considering similar laws.

Carol Ezzell

HISTORY OF SCIENCE

Meitner receives her due

Munich

FIFTY years after she participated in the discovery of nuclear fission and 21 years after her death, Lise Meitner is finally to be given full recognition for her achievements in West Germany. After years of lionizing her co-worker Otto Hahn, the Deutsches Museum in Munich agreed last week to give Lise Meitner equal credit in its portrayal of the discovery. Hahn, Meitner and Fritz Strassmann collaborated in the discovery of the fission of uranium in Berlin in 1938–39, for which Hahn won the 1944 Nobel prize. Hahn and Meitner both died in 1968 at the age of 89.

On the first floor of the Deutsches Museum, a laboratory table containing an apparatus for the neutron irradiation of uranium, which was thought to be used in the discovery, is labelled "worktable of Otto Hahn". But the apparatus was

participant. And it proved to be the last straw in a long series of complaints about the caption. Immediately afterwards, West German historian Barbara Orland of West Berlin circulated a petition asking for a change to the caption.

Sime explains that Meitner, in a letter dated 21 December 1938, encouraged Hahn to believe the unbelievable result that the uranium nucleus was not growing when it was bombarded by slow neutrons, as they had thought earlier, but was splitting into smaller nuclei like barium, with the potential release of tremendous amounts of energy. Hahn, a chemist, would later deny that the science of physics had contributed to the discovery. But on 27 December 1938, he added a paragraph on fission to the page proofs of a paper in the journal *Die Naturwissenschaften*. Meitner and her nephew Otto R. Frisch published a theoretical paper on

the phenomenon in *Nature* a few months later (143, 239; 1939).

Hahn, one of a handful of non-Nazis in his institute, was fearful of losing his chair to a rival. He denied having consulted with Meitner in order to protect himself from attacks by physicists who had remained in the institute after Meitner had left. According to Sime, Meitner was badly hurt when Hahn continued to deny her role after the war.

Hahn became one of the few scientific heroes in a demoralized postwar Germany. After returning from internment in Britain in 1946, Hahn (who became the first president of the Max Planck Society) worked hard to ensure that German science rose from the ashes. As West German historian Fritz Krafft has pointed out, Hahn's version of the discovery came to dominate all others, and something of a personality cult grew up around Hahn among his pupils and collaborators.

Mayr, who disputes the existence of a Hahn personality cult, says he nominated Meitner for the museum's "Hall of Fame" in February, before the latest row. The hall contains busts of 38 "German" scientific greats, from Copernicus (a Pole) to Einstein. He expects her to be installed next May. Meitner, who found it to be "half a crime to be a woman" in a Swedish scientific laboratory, says Sime, will thus gain admittance, albeit posthumously, to at least one exclusive club. All other members of the hall are male.

Steven Dickman

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Deutsches Museum, Munich, has promised that the label on "Hahn's" laboratory table will be altered to give equal recognition to Meitner.

designed by Meitner in the physics section of the Kaiser Wilhelm Institute for Chemistry before she had to flee to Sweden in 1938. Meitner, an Austrian Jew, feared dismissal by the Nazis. A caption describes Meitner as a "Mitarbeiterin", which literally means "co-worker" but in German carries the implied meaning of "subordinate". Meitner was not mentioned at all until the caption was rewritten in February 1989 on the orders of museum director Otto Mayr. Mayr says the museum will change the label to give equal recognition to Meitner.

Much of the credit for the reassessment of Meitner must go to Ruth Sime, a historian from Sacramento, California. Sime, who is writing the first scholarly biography of Meitner, presented a paper on Meitner last week at a congress for historians of science that took place, ironically, in the Deutsches Museum itself.

The presentation by Sime received "the closest thing to a standing ovation I have ever seen at a congress", said one

A definition of entropy

SIR—It is not easy to accept Charles Kittel's remarks (*Nature* 339, 170; 1989) concerning the definition of entropy. He objects that "no dictionary has advanced beyond the age of steam". While one may sympathize with his regrets that expositions of classical thermodynamics are often still written in a manner in keeping with the spirit of the industrial revolution, I reject his apodeictic definition of entropy as "the logarithm of the number of quantum states accessible to a system". First and foremost, entropy — like the second law of thermodynamics whence it arises — is about irreversibility. The logarithm of a number of quantum states, however, in no way suggests to the uninitiated any connection with this fundamental phenomenon; nor indeed is the irreversibility of natural processes easily established in reductionist theories. Moreover, Kittel's preferred definition

obviously has no place in phenomenological thermodynamics. Does this not imply that this powerful discipline is to be abandoned?

In short, I firmly believe that the ordinary, non-specialist dictionary should give pride of place to a contemporary phenomenological definition of entropy. Such a definition will reflect the existence of an ordering amongst the states of any adiabatically isolated system. On the lowest technical level it might be something like this: "the entropy of a system [in any given state] is a number [attached to that state] such that the relative adiabatic accessibility of any two states is governed by the difference between their entropies"; that is, an adiabatic transition from any initial state to any final state of lower entropy is impossible. (The assertion that such a labelling exists then amounts to a statement of the second law.) That one

has here in the first instance a definition of empirical rather than of metrical entropy is of no concern in the present context.

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What is truth?

SIR—An unfortunate impression may have been given by your leading article Can heresy be real?!. Your somewhat ambiguous sentence "[the] standard complaint against the popperian view of science — that there is no such thing as truth — can also be turned inside out, to show that, in the fields in which hypotheses cannot yet be tested, there is no such thing as being wrong" could easily be understood to mean that, according to Popper, there is no such thing as truth. No-one who knows Popper's work could think so; but people sometimes do think so, as was shown by another recent article in *Nature*², where he was attacked, again ambiguously, as a "betrayal of the truth". The doctrine that truth does exist, and is the aim of science, has been held by Popper, following Tarski³, since 1935; yet it is hardly more popperian than the doctrine that the Earth is not flat. What is a distinctively popperian thesis is that we devise tests for hypotheses because they may be wrong (false), not to prove them right (true) and that by trying to eliminate error we may with luck (but never with certainty) latch on to some part of the objective and absolute truth.

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1. *Nature* 340, 82 (1989).

2. Theoharis, T. & Psimopoulos, M. *Nature* 329, 595–598 (1987).

3. Tarski, A. in *Logic, Semantics, Metamathematics*, 152–278 (Oxford University Press, 1956).

Unstatistical bias

SIR—Some of the British research councils calculate, for each university department, the proportion of students they support who complete their theses within four years. Those departments in which a certain percentage (currently the Economic and Social Research Council specify 35 per cent, Natural Environment Research Council 45 per cent, Science and Engineering Research Council 40 per cent) of the students fail to complete are 'black-listed' and not given further studentships. This policy initially seems reasonable but may contain some major statistical injustices.

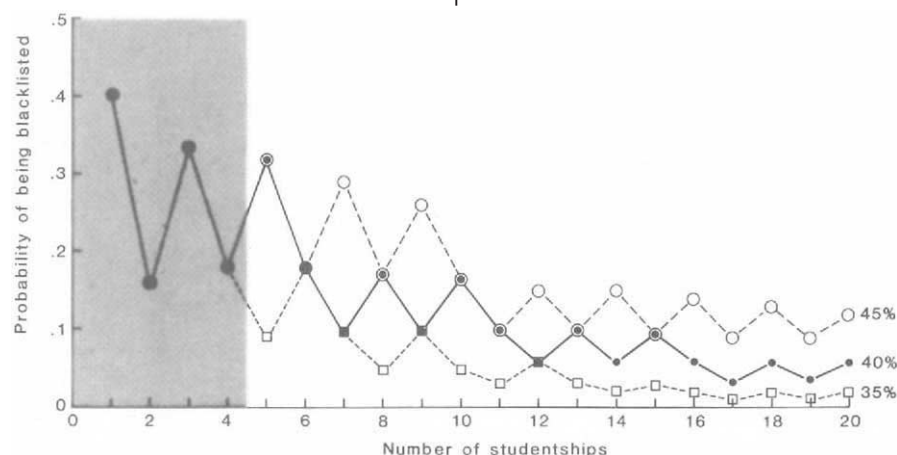
The issue can be considered by comparing two scenarios: (1) Some departments are lazy, inefficient and have low morale. Their students are slow in writing up and few complete within four years. Blacklisting would encourage such departments to

mend their ways. (2) There is little real difference between departments. Due to chance events, there will be variation in the proportion of students completing their theses and some departments will have a higher success rate than others. With this scenario, blacklisting is demoralizing, unjust and shows an ignorance of statistics.

The probability of being blacklisted can be calculated from the binomial distribution, assuming that the average completion rate is 60 per cent. The relationship between this probability and department size is shown in the figure — most departments have fewer than ten studentships. This analysis shows that there are erratic differences between different sized departments and that there is considerably less risk for large departments.

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The probability of being blacklisted by chance for departments with different numbers of studentships. The three lines show blacklisting at three different thresholds. Departments with fewer than five studentships are excluded from blacklisting and are shaded.

Cambridge reviews

SIR—You refer (*Nature* 339, 412; 1989) to "the independent review group set up to examine [Cambridge] University's management", but in fact the syndicate chaired by Sir Douglas Wass was appointed 'to consider the government of the University', a related but different question. The distinction is important, all the more so under a government that would like to see it disappear.

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The decline of science in England

Charles Babbage

Nothing but the full expression of public opinion can remove the evils that chill the enthusiasm, and cramp the energies of the science of England.

If anyone shall endeavour to account for the opinions stated in these pages by ascribing them to any imagined circumstance peculiar to myself, I think he will be mistaken. That science has long been neglected and declining in England is not an opinion originating with me, but is shared by many, and has been expressed by higher authority than mine.

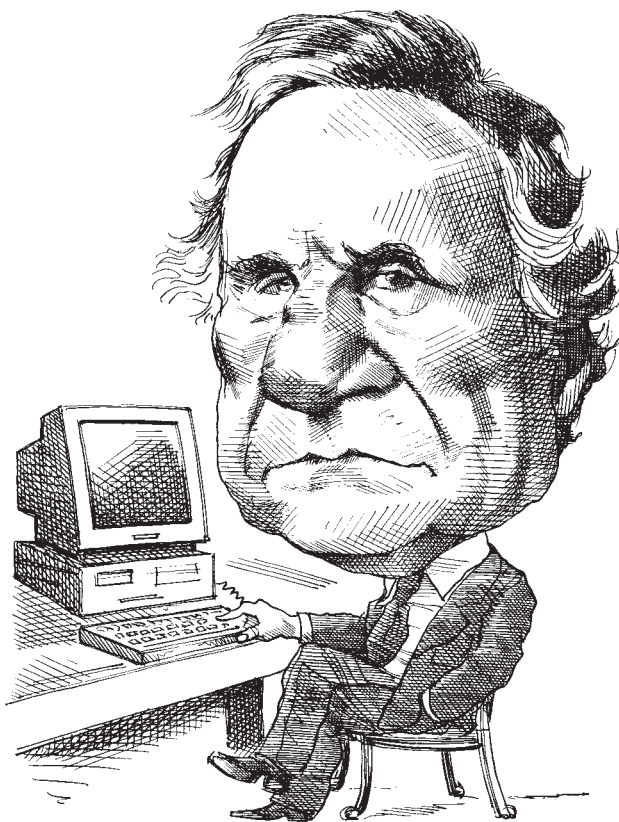
Many attacks have lately been made on the conduct of various scientific bodies and of their officers, and severe criticism has been lavished upon some of their productions. Odium has been cast upon some of these for being anonymous. If a fact is to be established by testimony, anonymous assertion is of no value; if it can be proved, by evidence to which the public have access, it is of no consequence (for the cause of truth) who produces it. A matter of opinion derives weight from the name which is attached to it; but a chain of reasoning is equally conclusive, whoever may be its author.

Perhaps it would be better for science that all criticism should be avowed. It would certainly have the effect of rendering it more matured and less severe; but, on the other hand, it would have the evil of frequently repressing it altogether, because there exists amongst the lower ranks of science, a *genus irritabile*, who are disposed to argue that every criticism is personal. It is clearly the interest of all who fear enquiries to push this principle as far as possible, whilst those whose sole object is truth can have no apprehensions from the severest scrutiny. There are few circumstances which so strongly distinguish the philosopher as the calmness with which he can reply to criticisms he may think undeservedly severe.

Education

That the state of knowledge in any country will exert a directive influence on the general system of instruction adopted in it, is a principle too obvious to require investigation. It is, therefore, not unreasonable to suppose that some portion of the neglect of science in England may be attributed to the system of education we pursue.

Much more might be said on a subject so important to the interests of the country, but my wish is merely to open it for our own consideration and discussion. We have already done so much for the improvement of our system of instruction that public opinion will not reproach us for any unwillingness to alter. It is our first



Charles Babbage, drawn by David Smith

duty to be well satisfied that we can improve: such alterations ought only to be the result of a most mature consideration, and of a free interchange of sentiments on the subject, in order that we may condense upon the question the accumulated judgement of many minds.

It is in some measure to be attributed to the defects of our system of education that scientific knowledge scarcely exists among the higher classes of society. The discussions in the House of Lords or the House of Commons which arise on the occurrence of any subjects connected with science, sufficiently prove this fact — which, if I had consulted the extremely limited nature of my own personal experience, I should, perhaps, have doubted.

Inducements

Professional impulses. A large portion of those who are impelled by ambition or necessity to advance themselves in the world, make choice of some profession in which they imagine their talents likely to be rewarded with success; and there are peculiar advantages resulting to each from this classification of society into professions. The *esprit de corps* frequently overpowers the jealousy which exists between individuals, and pushes on to advantageous situations some of the more fortunate of the profession; whilst, on the other hand, any injury or insult offered to the weakest is redressed or resented by the whole body.

The pursuit of science does not, in England, constitute a distinct profession, as it does in many other countries. It is therefore, on that ground alone, deprived of many of the advantages which attach to professions. One of its greatest misfortunes arises from this circumstance; for the subjects on which it is conversant are so difficult, and require such unremitted devotion of time, that few who have not spent years in their study can judge of the relative knowledge of those who pursue them. It follows, therefore, that the public, and even that men of sound sense and discernment, can scarcely find means to distinguish between the possessors of knowledge, in the present day, merely elementary, and those whose acquirements are of the highest order.

In England, the profession of the law is that which seems to hold out the strongest attraction to talent, from the circumstance, that in its ability, coupled with exertion, even though unaided by patronage, cannot fail of obtaining reward. It is frequently chosen as an introduction to public life. It also presents great advantages, from its being a qualification for many situations more or less remotely connected with it, as well as from the circumstance that several of the highest officers of the state must necessarily have sprung from its ranks.

A powerful attraction exists, therefore, to the promotion of a study and of duties of all others engrossing the time most

completely, and which is less benefitted than most others by any acquaintance with science. This is one among the causes why it so very rarely happens that men in public situations are at all conversant even with the commonest branches of scientific knowledge, and why scarcely an instance can be cited of such persons acquiring a reputation by any discoveries of their own.

National encouragement. The little encouragement which at all previous periods has been afforded by the English Government to the authors of useful discoveries, or of new and valuable inventions, is justified on the following grounds:

1. The public, who consume the new commodity or profit by the invention, are much better judges of its merit than the government can be.

2. The reward which arises from the sale of the commodity is usually much larger than that which government would be justified in bestowing; and it is exactly proportioned to the consumption, that is, to the want which the public feel for the new article.

It must be admitted that, as general principles, these are correct: there are, however, exceptions which flow necessarily from the very reasoning from which they were deduced. Without entering minutely into these exceptions, it will be sufficient to show that all abstract truth is entirely excluded from reward under this system. It is only the application of principles to common life which can be thus rewarded. A few instances may perhaps render this position more evident. The principle of the hydrostatic paradox was known as a speculative truth in the time of Stevinus (about 1600); and its application to raising heavy weights had long been stated in elementary treatises on natural philosophy, as well as constantly exhibited in lectures. Yet it may fairly be regarded as a mere abstract principle until the late Mr Bramah, by substituting a pump instead of the smaller column, converted it into a most valuable and powerful engine. The principle of the convertibility of the centres of oscillation and suspension in the pendulum, discovered by Huygens more than a century and a half ago, remained, until within these few years, a sterile though most elegant proposition; when, after being hinted at by Prony, and distinctly pointed out by Bonenberger, it was employed by Captain Kater as the foundation of a most convenient practical method of determining the length of the pendulum.

Other instances might, if necessary, be adduced, to show that long intervals frequently elapse between the discovery of new principles in science and their practical application: nor ought this at all to surprise us. Those intellectual qualifications which give birth to new principles or to new methods are of quite a different

order from those which are necessary for their practical application.

At the time of the discovery of the beautiful theorem of Huygens, it required in its author not merely a complete knowledge of the mathematical science of his age, but a genius to enlarge its boundaries by new creations of his own. Such talents are not always united with a quick perception of the details, and of the practical applications of the principles they have developed; nor is it for the interest of mankind that minds of this high order should lavish their powers on subjects unsuited to their grasp.

On the other hand, for one person who is blessed with the power of invention, many will always be found who have the capacity of applying principles; and much of the merit ascribed to these applications will always depend on the care and labour bestowed in the practical detail.

If, therefore, it is important to the country that abstract principles should be applied to practical use, it is clear that it is also important that encouragement should be held out to the few who are capable of adding to the number of those truths on which such applications are founded. Unless there exist peculiar institutions for the support of such enquirers, or unless the government directly interfere, the contriver of a thaumatrope may derive profit from his ingenuity, whilst he who unravels the laws of light and vision, on which multitudes of phenomena depend, shall descend unrewarded to the Tomb.

Perhaps it may be urged that sufficient encouragement is already afforded to abstract science in our different universities, by the professorships established at them. It is not however in the power of such institutions to create; they may foster and aid the development of genius; and, when rightly applied, such stations ought to be its fair and honourable rewards. In many instances their emolument is small; and, when otherwise, the lectures which are required from the professor are not perhaps in all cases the best mode of employing the energies of those who are capable of inventing.

Where would have been the military renown of England, if, with an equally improvident waste of mental power, its institutions had forced the Duke of Wellington to employ his life in drilling recruits instead of planning campaigns?

Prospects. Let us now look at the prospects of a young man at his entrance into life, who, impelled by an almost irresistible desire to devote himself to the abstruser sciences, or who, confident in the energy of youthful power, feels that the career of science is that in which his mental faculties are most fitted to achieve the reputation for which he pants. What are his prospects? Can even the glowing pencil of enthusiasm add colour to the blank before

him? There are no situations in the state; there is no position in society to which hope can point, to cheer him in his laborious path. If, indeed, he belong to one of our universities, there are some few chairs in his *own* Alma Mater to which he may at some distant day pretend; but these are not numerous; and whilst the salaries attached are seldom sufficient for the sole support of the individual, they are very rarely enough for that of a family. What then can he reply to the entreaties of his friends, to betake himself to some business in which perhaps they have power to assist him, or to choose some profession in which his talents may produce for him their fair reward? If he have no fortune, the choice is taken away: he *must* give up that line of life in which his habits of thought and his ambition qualify him to succeed eminently, and he *must* choose the bar or some other profession, in which, among so many competitors, in spite of his great talents, he can be but moderately successful. The loss to him is great, but to the country it is greater. We thus, by a restrictive misapplication of talent which our institutions create, exchange a profound philosopher for but a tolerable lawyer.

The Royal Society

Becoming a Fellow. I have no intention of stating what *ought* to be the qualifications of a Fellow of the Royal Society; but, for years, the practical mode of arriving at that honour has been as follows:

A.B. gets any three Fellows to sign a certificate, stating that he (A.B.) is desirous of becoming a member, and likely to be a useful and valuable one. This is handed in to the Secretary, and suspended in the meeting-room. At the end of ten weeks, if A.B. has the good fortune to be perfectly unknown by any literary or scientific achievement, however small, he is quite sure of being elected as a matter of course. If, on the other hand, he has unfortunately written on any subject connected with science, or is supposed to be acquainted with any branch of it, the members begin to enquire what he has done to deserve the honour; and, unless he has powerful friends, he has a fair chance of being blackballed.

Several times, while I have been consulting books or papers at Somerset House, persons have called to ask the Assistant Secretary the mode of becoming a member of the Royal Society. I should conjecture, from some of these applications, that it is not very unusual for gentlemen in the country to order their agents in London to take measures for putting them up at the Royal Society.

Another circumstance worthy of the attention of the Society is to consider whether it is desirable, except in special cases, to have military persons appointed to any of its offices. There are several

peculiarities in the military character, which, though they do not absolutely unfit their possessors for the individual prosecution of science, may in some degree disqualify such persons from holding offices in scientific institutions. The habits both of obedience and command, which are essential in military life, are little fitted for that perfect freedom which should reign in the councils of science. If a military chief commit an oversight or an error, it is necessary, in order to retain the confidence of those he commands, to conceal or mask it as much as possible. If an experimentalist make a mistake, his only course to win the confidence of his fellow labourers in science, and to render his future observations of any use, is to acknowledge it in the most full and explicit manner.

Causes of the present state of the Royal Society. The Society has for years been managed by a *party*, or *coterie*, or by whatever other name may be most fit to designate a combination of persons, united by no expressed compact or written regulations, but who act together from a community of principles. That each individual has invariably supported all the measures of the *party*, is by no means the case; and whilst instances of opposition among them have been very rare, a silent resignation to circumstances has been the most usual mode of meeting measures they disapproved. The great object of this, as of all other parties, has been to maintain itself in power, and to divide, as far as it could, all the good things among its members. It has usually consisted of persons of very moderate talent, who have had the prudence, whenever they could, to associate with themselves other members of greater ability, provided these latter would not oppose the *system*, and would thus lend to it the sanction of their name.

Of the arguments employed by those who support the *system of management* by which the Royal Society is governed, I shall give a few samples: refutation is rendered quite unnecessary — juxtaposition is alone requisite. If any member, seeing an improper appointment in contemplation, or any abuse in the management of the affairs of the Society continued, raise a voice against it, the ready answer is, Why should you interfere? It may not be quite the thing you approve; but it is no affair of yours. If, on the other hand, it do relate to himself, the reply is equally ready. It is immediately urged: The question is of a personal nature; *you* are the last person who ought to bring it forward; *you* are *yourself* interested. If any member of the Society, feeling annoyed at the neglect, or hurt by the injuries or insults of the Council, show signs of remonstrance, it is immediately suggested to him that he is irritated, and ought to wait until his feelings subside,

“Scientific enquiries are more exposed than most others to the inroads of pretenders. I feel that I shall deserve the thanks of all who really value truth, by stating some of the methods of deceiving practised by unworthy claimants for its honours, whilst the mere circumstance of their arts being known may deter future offenders.”

and he can judge more coolly on the subject; whilst with becoming candour they admit the ill-treatment, but urge forbearance. If, after an interval, when reflection has had ample time to operate, the offence seems great as at first, or the insult appears unmitigated by any circumstances on which memory can dwell — if it is then brought forward, the immediate answer is, the affair is out of date — the thing is gone by — it is too late to call in question a transaction so long past.

In the same spirit I have heard errors of calculation or observation defended. If small errors occur, it is said that they are too trifling to be of any importance. If larger errors are pointed out, it is immediately contended that they can deceive nobody, because of their magnitude. Perhaps it might be of some use, if the Council would oblige the world with their *scale of error*, with illustrations from some of the most *recent* and *approved* works, and would favour the uninformed with the orthodox creed upon all grades, from that which baffles the human faculties to detect up to that which becomes innocuous from its size.

Observations

Minute precision. No person will deny that the highest degree of attainable accuracy is an object to be desired, and it is generally found that the last advances towards precision require a greater devotion of time, labour, and expense, than those which precede them. The first steps in the path of discovery, and the first approximate measures, are those which add most to the existing knowledge of mankind.

The extreme accuracy required in some of our modern enquiries has, in some respects, had an unfortunate influence by favouring the opinion that no experiments are valuable unless the measures are most minute, and the accordance among them most perfect.

This desire for extreme accuracy has called away the attention of experimenters from points of far greater importance; it seems to have been too much overlooked in the present day that genius marks its tract not by the observation of quantities inappreciable to any but the acutest senses, but by placing nature in

such circumstances that she is forced to record her minutest variations on so magnified a scale that an observer, possessing ordinary faculties shall find them legibly written. He who can see portions of matter beyond the ken of the rest of his species, confers an obligation on them, by recording what he sees; but their knowledge depends both on his testimony and on his judgement. He who contrives a method of rendering such atoms visible to ordinary observers, communicates to mankind an instrument of discovery, and stamps his own observations with a character alike independent of testimony or of judgement.

The frauds of observers. Scientific enquiries are more exposed than most others to the inroads of pretenders. I feel that I shall deserve the thanks of all who really value truth, by stating some of the methods of deceiving practised by unworthy claimants for its honours, whilst the mere circumstance of their arts being known may deter future offenders.

There are several species of impositions that have been practised in science, which are but little known, except to the initiated and which it may perhaps be possible to render quite intelligible to ordinary understandings. These may be classed under the heads of hoaxing, forging, trimming, and cooking.

Of hoaxing. This, perhaps, will be better explained by an example. In the year 1788, M. Gioeni, a knight of Malta, published at Naples an account of a new family of Testacea, of which he described, with great minuteness, one species, the specific name of which has been taken from its *habitat*, and the generic he took from his own family, calling it Gioenia Sicula. It consisted of two rounded triangular valves, united by the body of the animal to a smaller valve in front. He gave figures of the animal and of its parts; described its structure, its mode of advancing along the sand, the figure of the tract it left, and estimated the velocity of its course at about two-thirds of an inch per minute. He then described the structure of the shell, which he treated with nitric acid, and found it approach nearer to the nature of bone than any other shell.

The editors of the *Encyclopédie Methodique*, have copied this description, and have given figures of the Gioenia Sicula. The fact, however, is that no such animal exists, but that the knight of Malta, finding on the Sicilian shores the three internal bones of one of the species of Bulla, of which some are found on the south-western coast of England, described and figured these bones most accurately, and drew the whole of the rest of the description from the stores of his own imagination.

Such frauds are far from justifiable; the only excuse which has been made for them is when they have been practised on

scientific academies which had reached the period of dotage.

Forging differs from hoaxing, inasmuch as in the latter the deceit is intended to last for a time, and then be discovered, to the ridicule of those who have credited it; whereas the forger is one who, wishing to acquire a reputation for science, records observations which he has never made. This is sometimes accomplished in astronomical observations by calculating the time and circumstances of the phenomenon from tables. The observations of the second comet of 1784, which was only seen by the Chevalier D'Angos, were long suspected to be a forgery, and were at length proved to be so by the calculations and reasonings of Encke. The pretended observations did not accord among each other in giving any possible orbit. But M. Encke detected an orbit, belonging to some of the observations, from which he found that all the rest might be almost precisely deduced, provided a mistake of a unity in the index of the logarithm of the radius vector were supposed to have been made in all the rest of the calculations.

Fortunately instances of the occurrence of forging are rare.

Trimming consists in clipping off little bits here and there from those observations which differ most in excess from the mean, and in sticking them on to those which are too small; a species of 'equitable adjustment', as a radical would term it, which cannot be admitted in science.

This fraud is not perhaps so injurious (except to the character of the trimmer) as cooking, which the next paragraph will teach. The reason of this is that the *average* given by the observations of the trimmer is the same, whether they are trimmed or untrimmed. His object is to gain a reputation for extreme accuracy in making observations; but from respect for truth, or from a prudent foresight, he does not distort the position of the fact he gets from nature, and it is usually difficult to detect him. He has more sense or less adventure than the cook.

Of cooking. This is an art of various forms, the object of which is to give to ordinary observations the appearance and character of those of the highest degree of accuracy.

One of its numerous processes is to make multitudes of observations, and out of these to select those only which agree, or very nearly agree. If a hundred observations are made, the cook must be very unlucky if he cannot pick out fifteen or twenty which will do for serving up.

Another approved receipt, when the observations to be used will not come within the limit of accuracy, which it has been resolved they shall possess, is to calculate them by two different formulae. The difference in the constants employed in those formulae has sometimes a most happy effect in promoting unanimity

among discordant measures. If still greater accuracy is required, three or more formulae can be used.

It must be admitted that this receipt is in some instances rather hazardous: but in cases where the positions of stars, as given in different catalogues, occur, or different tables of specific gravities, specific heats, etc., it may safely be employed. As no catalogue contains all stars, the computer must have recourse to several; and if he is obliged to use his judgement in the selection, it would be cruel to deny him any little advantage which might result from it. It may, however, be necessary to guard against one mistake into which persons might fall.

If an observer calculates particular stars from a catalogue which makes them accord precisely with the rest of his results, whereas had they been computed from other catalogues the difference would have been considerable, it is very unfair to accuse him of *cooking*; for — those catalogues may have been notoriously inaccurate; or — they may have been superseded by others more recent, or made with better instruments; or — the observer may have been totally ignorant of their existence.

It sometimes happens that the constant quantities in formulae given by the highest authorities, although they differ among themselves, yet they will not suit the materials. This is precisely the point in which the skill of the artist is shown; and an accomplished cook will carry himself triumphantly through it, provided happily some mean value of such constants will fit his observations. He will discuss the relative merits of formulae he has just knowledge enough to use; and with admirable candour assigning their proper share of applause to Bessel, to Gauss, and to Laplace, he will take *that* mean value of the constant used by three such philosophers, which will make his own observations accord to a miracle.

There are some few reflections which I would venture to suggest to those who cook, although they may perhaps not receive the attention which in my opinion they deserve, from not coming from the pen of an adept.

In the first place, it must require much time to try different formulae. In the next place it may happen that, in the progress of human knowledge, more correct formulae may be discovered, and constants may be determined with far greater precision. Or it may be found that some physical circumstance influences the results (although unsuspected at the time), the measure of which circumstance may perhaps be recovered from other contemporary registers of facts. Or if the selection of observations has been made with the view of its agreeing precisely with the latest determination, there is some little danger that the average of the whole

may differ from that of the chosen ones, owing to some law of nature, dependent on the interval between the two sets, which law some future philosopher may discover, and thus the very best observations may have been thrown aside.

In all these, and in numerous other cases, it would most probably happen that the cook would procure a temporary reputation for unrivalled accuracy at the expense of his permanent fame. It might also have the effect of rendering even all his crude observations of no value; for that part of the scientific world whose opinion is of most weight, is generally so unreasonable as to neglect altogether the observations of those in whom they have, on any occasion, discovered traces of the artist. In fact, the character of an observer, as of a woman, if doubted is destroyed.

Advancing science in England

Among the various proposals for encouraging science, the institution of an order of merit has been suggested. It is somewhat singular that whilst in most of the other kingdoms of Europe such orders exist for the purpose of rewarding, by honorary distinctions, the improvers of the arts of life, or successful discoverers in science, nothing of the kind has been established in England.

Our orders of knighthood are favourable only to military distinction. It has been urged, as an argument for such institutions, that they are a cheap mode of rewarding science, whilst, on the other hand, it has been objected that they would diminish the value of such honorary distinctions by making them common. Another objection, however, appears to me to possess far greater weight; and however strong the disposition of the government might be (if such an order existed) to fill it properly, I do not believe that, in the present state of public opinion respecting science, it could be done. In all probability, it would be filled up through the channels of patronage, and by mere jobbers in science.

Another proposal of a similar kind has also been talked of, one which it may appear almost ridiculous to suggest in England, but which would be considered so in no other country. It is to ennoble some of the greatest scientific benefactors of their country. But here, again, until there existed some knowledge of science among the higher classes, and a sound state of public opinion relative to science, the execution of the plan could only be injurious. □

Charles Babbage was Lucasian Professor of Mathematics in the University of Cambridge. This article consists of edited extracts from Reflections on the Decline of Science in England and on Some of its Causes, published in 1830 and reprinted as Vol. 7 of The Works of Charles Babbage (see the book review on p.517 of this issue of Nature).

Neutrons aged in the bottle

Knowing the half-life of the neutron is important for particle physicists and cosmologists alike. A new technique should soon improve the accuracy of measurements to better than the present half per cent.

THE value of the fine-structure constant of electromagnetic theory is known to an accuracy of about one part in ten million. In recent measurements of the magnetic moment of the electron, whose small departure from the classical value constitutes a powerful diagnostic test of quantum electrodynamics, the error comes only in the tenth decimal place. So it may come as something of a surprise to discover that the best current estimate of the lifetime of the neutron, which was the third 'elementary' particle to be discovered, is 891.6 seconds with an error of ± 5.1 seconds — an accuracy of no better than half a per cent.

The error should not be taken as indicative of any lack of effort on the part of experimenters. Measurements of the neutron's half-life are constantly being attempted by groups all around the world, and the magnitude of the 5-second error represents more than anything else a measure of the difficulty that these different groups have in coming up with consistent values. On the face of it, a new value given in the 7 August issue of *Physical Review Letters* (63, 593; 1989) by W. Mamepe *et al.*, five physicists from France, the United Kingdom and the United States, does not improve things markedly: their new estimate is 887.6 ± 3 seconds.

This is certainly consistent with the previous best guess, but the error is much the same. What is of interest in the new measurement is the manner in which it was obtained, using stored ultracold neutrons, and the promise it holds for future determinations.

The neutron decays, when not bound up in a stable nucleus, into a proton, an electron and a neutrino. As such, it can be considered the fundamental manifestation of radioactive beta-decay, and therein lies its significance for elementary particle physics. Enrico Fermi, before he moved to the United States and created the first self-sustaining nuclear reaction, was responsible for a theoretical analysis of beta decay that survives today in standard accounts of weak interaction theory.

Fermi introduced a new strength parameter, which measured the magnitude of the then-unknown interaction responsible for beta decay. This kind of nuclear instability was later interpreted as evidence of a new force of nature, the weak interaction, which has itself now been subsumed into the unified electroweak interaction of Glashow, Salam and Weinberg.

Nevertheless, Fermi's constant, or a slightly different formulation of it, has remained as the fundamental constant of the weak interaction, as Newton's constant remains the basic parameter of Einstein's theory of gravity. And measurement of the neutron's half-life remains one of the few comparatively simple ways for experimenters to get at the value of this number. (Radioactive half-lives of large nuclei can be measured with good accuracy by obtaining a lump of the appropriate substance and counting decay products, but the relation of the weak interaction strength to measured life-time is obscured by the complication of many-body nuclear physics.)

On top of its intrinsic importance to fundamental physics, the value of the half-life of the neutron also has an anthropic significance in cosmology. At about one second old, the Universe consists of protons, neutrons, electrons and radiation, with protons and neutrons having roughly equal numbers.

The radiation temperature is then too high to allow protons and neutrons to get together as deuterium nuclei and thence form heavier elements, but after three minutes, deuterium can survive and nucleosynthesis can proceed.

To a first approximation, all the neutrons that are around at a cosmic age of three minutes end up in 'He' nuclei. If the neutron were stable, and at this critical age were still as abundant as protons, then every two neutrons would team up with a pair of protons to form a 'He' nucleus, and nothing would be left over. But the neutron's instability reduces its numbers enough, by the time 180 seconds have been ticked off, that only about a quarter of the Universe's raw material is cooked into 'He', leaving enough hydrogen around, along with a few other trace elements, for stars and galaxies to begin forming. As a nuclear fuel, hydrogen is considerably more inflammable than helium, and stellar evolution is able ultimately to manufacture the heavy elements of which planets and their inhabitants are made.

The imprecision with which the neutron's half-life is known is thus no reflection of the urgency of the need to know. The difficulties are practical ones. A standard technique in many earlier experiments was to measure the rate at which a beam of neutrons emitted electrons. Counting electrons is easy: charged

particles can be siphoned off with suitable magnetic fields, and detectors are efficient. But to determine a decay constant from such an experiment, the number of neutrons needs also to be known, and this is the source of inaccuracy. Neutron detectors are not very efficient, and because the motion of neutrons cannot be controlled by electromagnetic fields, particles can drift out of the beam without the experimenter being able to tell.

In the technique used by W. Mamepe and P. Ageron of the Institut Laue-Langevin in Grenoble, France, and their colleagues, a source of ultracold neutrons (with kinetic energies less than one micro electron volt) is used to fill a neutron 'bottle'. To measure the half-life, the number of neutrons has to be measured going in, and at some later time, and as the same detector is used for both measurements the lack of detector efficiency becomes irrelevant.

But this method, which has become possible only with the advent of ultracold neutron sources during the past couple of years, still requires a good deal of delicacy. The neutrons are confined to a small volume, some tens of centimetres on a side, made of glass plates coated with a viscous heavy oil which both seals in the vacuum and reflects neutrons with high efficiency.

The rate at which neutrons are lost is a combination of decays and absorption by the walls, and the latter must be estimated and subtracted to yield a true half-life. Moreover, during the several thousand seconds through which the neutrons are confined, collisions with the oil-coated walls gradually reduce the average energy of the neutrons, and gravity causes them to settle slowly to the bottom. As the experiment proceeds, the physical distribution of the neutrons in the glass box changes, which introduces a systematic error in neutron counts taken at different times. This indeed proves to be the biggest source of error in the measurement.

But because this problem is fairly simple to understand, it is susceptible to revised experimental design and more sophisticated calculations of the loss rate. Undoubtedly other errors will intrude as the experiment is improved, but what Mamepe *et al.* have demonstrated is that a straightforward realization of this technique is at least as good as the most refined implementations of earlier methods.

David Lindley

Perfect quasicrystals?

D. P. DiVincenzo

QUASICRYSTALS are crystalline solids exhibiting icosahedral symmetry. Their discovery in Al-Mn alloys in 1984 caused great surprise, because the classical laws of crystallography forbid icosahedral symmetry in periodic crystals. This discovery required there to be a new kind of crystalline order, quasiperiodicity, which had rarely been considered before except by mathematicians. There was speculation that quasicrystals would qualify as a new phase of matter, with unique properties unknown to other crystalline solids, such as fractal electronic behaviour. Unfortunately, the properties of the dozens of real icosahedral alloys discovered subsequently were dominated by disorder and no novel phenomena were uncovered. Thus, excitement has been generated by the recent report by C. A. Guryan *et al.* (*Phys. Rev. Lett.* **62**, 2409–2412; 1989) who show that the icosahedral alloy, $\text{Al}_{65}\text{Cu}_{20}\text{Ru}_{15}$, is much less disordered, by the usual crystallographic criterion of the sharpness of X-ray diffraction lines, than any previous quasicrystal. Indeed, a recent study by P. A. Bancel at IBM, Yorktown Heights, shows that after annealing, the diffraction lines of the sister compound $\text{Al}_{65}\text{Cu}_{20}\text{Fe}_{15}$ are sharper than instrumental resolution — as good as the best conventional crystals. In short, these materials seem to be perfect quasicrystals, rekindling the search for unusual physical properties.

These developments give greater significance to important work on the mathematics of tilings done by Roger Penrose and others beginning in the 1970s. Penrose discovered a nonperiodic tiling of the plane, using two types of tiles, which, to use our present language, is a quasiperiodic crystal with pentagonal symmetry. He also found matching rules — local rules for how the two types of tiles may join — which force the quasiperiodicity; that is, the rules forbid both disordered tilings and ordered, periodic tilings. Later work by G. Y. Onoda and co-workers at IBM showed how the matching rules could be extended to a specific algorithm for growing the tiling from a seed. The resulting structure, the Penrose tiling, is unique and perfectly ordered in a mathematical sense.

Soon after the discovery of the quasicrystal alloys, three-dimensional generalizations of the Penrose tiling and the matching rules were found for icosahedral tilings. It became natural to assume that the alloys had atomic arrangements that correspond to the tile shapes in the three-dimensional Penrose tiling, and this was confirmed in studies of various alloy systems. Thus, there is general agreement that the new Al-Cu-Fe and Al-Cu-Ru alloys are actual physical realizations of

these mathematical constructions — they are icosahedral tilings with long-range quasiperiodic order. Other models, like Pauling's 'multiple twin' ideas, have been virtually ruled out.

Recent theoretical work, however, shows that there are other variants of the Penrose tiling model that are consistent with the data of Guryan *et al.*. In the original ideal Penrose tiling model, the dominant contribution to the free energy $F = U - TS$ would come from the energy U (T is temperature). It is hypothesized that the local atomic interactions enforce Penrose's matching rules, favouring the quasiperiodic ground state. Calculations by M. Widom *et al.* (*Phys. Rev. Lett.* **63**, 310–313; 1989) and K. Strandburg *et al.* (*Phys. Rev. Lett.* **63**, 314–317; 1989) show that the Penrose tiling can also be stabilized by the entropy S . If Penrose's matching rules are relaxed, the entropy is large because more varied packings of tiles are permitted. This has become known as the random-tiling model, although the randomness is very constrained by the requirements of icosahedral packing; the resulting state still has long-range icosahedral quasiperiodic order, despite the short-range disorder.

There is really no sharp distinction between the ideal Penrose tiling and this

random tiling. They represent two extremes of a continuous range of possibilities in which the quasicrystal is stabilized by a mixture of energy and entropy. Both models are based on the Penrose tiling, and both predict perfect long-range order as seen in the new alloys. However, it is important to determine which model most accurately describes $\text{Al}_{65}\text{Cu}_{20}\text{Ru}_{15}$ and $\text{Al}_{65}\text{Cu}_{20}\text{Fe}_{15}$, because it is only in the ideal-tiling limit that we would expect to find some of the truly unique features of the quasiperiodic state, like fractal properties in the electronic behaviour as described in the model of M. Kohmoto *et al.* (*Phys. Rev. B* **35**, 1020–1033; 1987).

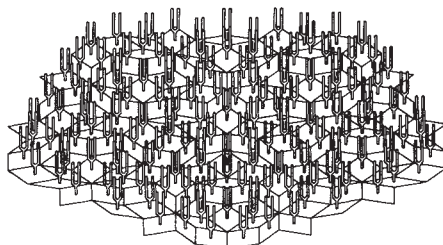
Further experiments are now being done that will help determine the version of the Penrose tiling that actually applies to the real materials. None of the results so far is conclusive, although some recent reports tend to support the random-tiling ideas. The electrical resistivity, as measured recently by A. P. Tsai *et al.* (*J. mat. Sci. Lett.* **8**, 253–256; 1989) and J. L. Wagner *et al.* (*Phys. Rev. B* **39**, 8091–8095; 1989) is fairly high, indicating a large degree of local disorder in these compounds. Also, recent X-ray diffraction studies by Bancel of Al-Cu-Fe at high temperatures indicate that the icosahedral phase is actually better ordered at elevated temperatures, which is consistent with entropy playing the dominant role in stability.

So it seems that researchers will have to seek further to find the truly perfect Penrose tiling, because even the new

In tune with quasiperiodicity

AS NOTED by DiVincenzo, quasiperiodicity can be expected to generate features entirely alien to normal periodic lattices. In an off-beat contribution to quasicrystal research, Shanjin He and J. D. Maynard have used 150 tuning forks arranged in a two-dimensional Penrose tiling to simulate the spectrum of electronic states in a quasicrystal (*Phys. Rev. Lett.* **62**, 1888–1891; 1989).

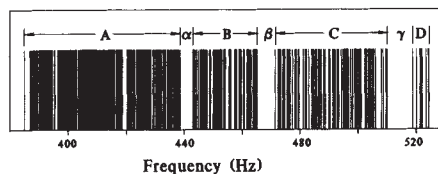
The tines of the tuning forks are joined to their nearest neighbours by arcs of steel wire. The connection with a real quasicrystal is that the forks make a coupled array of oscillators: the electrons in a lattice,



equally, comprise a coupled set of oscillators. By 'humming' at this pentagonal aeolian harp and listening — with an electric-guitar pickup — the authors effectively find the spectrum of frequencies at which the entire

ensemble vibrates — which is equivalent to revealing the eigenvalue spectrum of the electronic structure.

The striking outcome is that the resonant frequencies form a set of bands — marked



A, B, C and D in the spectrum — much as one finds for the states in a crystalline solid. But the gaps between the bands (α , β , γ) are in the ratio of the golden mean $(\sqrt{5} + 1)/2$ — the ratio of the areas of the two rhombi in the tiling pattern — and the bandwidths themselves are in the ratio of powers of this number. The observation of such ratios in a real quasicrystal might be indicative of perfect quasiperiodicity.

The patterns traced by the tines of the tuning forks, revealed by tiny mirrors fixed to their points, typically bear the 5-fold symmetry of the Penrose tiling. The wavefunctions of the electrons in the quasiperiodic potential of a quasicrystal could be expected to have this characteristic too. □

'perfect' quasicrystals are not really perfect. Of course, the very existence of icosahedral solids was only established five years ago, so further advances would not be surprising. The theoreticians' point of view is that since there is nothing in the laws of physics to forbid the appearance of

an ideal Penrose tiling in nature, their eventual discovery in the laboratory is inevitable. □

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CELL MOTILITY

Myosin linkage strengthened

Mark S. Mooseker

IN THE redoubtable double-act that underlies muscle action, actin's partner is a two-headed, tailed variety of myosin. But for cell motility, at least of amoeboid cells, that form of myosin is dispensable, and a single-headed, tail-less variety — myosin I — seems to take its place. Adams and Pollard, on page 565 of this issue¹, and Korn and co-workers^{2,3} now report evidence of interactions between the amoeboid cell surface and myosin I that strongly support the role that has been promoted for it.

Because the slime mould, *Dictyostelium*, contains 'conventional' myosin (of the two-headed type now called myosin II), it came as a considerable surprise that the disruption of myosin, either genetically^{4,5} or by injection of inhibitory antibodies⁶ had little effect on the motility of *Dictyostelium* amoeba. They can still move, extend pseudopods and undergo phagocytosis, although normal translocation of cells is somewhat impaired^{4,5}, and cytokinesis is inhibited — a result that confirms earlier studies on cleavage in echinoderm embryos. There was no doubt that actin filaments participate in all these motile phenomena, but what is the force generator?

First characterized in *Acanthamoeba* and later in *Dictyostelium*, myosins I are single-headed and lack the carboxy-terminal, α -helical tail domain of myosins II (see ref. 7). Instead of a tail, *Acanthamoeba* myosins IA and IB each have a non- α -helical domain that contains a second actin-binding site, which unlike the binding site on the head domain of all myosins, is ATP-insensitive. It is this second site that gives myosin I the potential to crosslink filaments and generate force.

Adams and Pollard¹ now provide evidence that *Acanthamoeba* myosin I can interact directly with the phospholipid bilayer via interaction with anionic phospholipids. They show that myosin I binds with high affinity both to NaOH-stripped *Acanthamoeba* plasma membrane vesicles and to phospholipid vesicles composed of either phosphatidyl serine or phosphatidyl inositol 4,5-bisphosphate, but not to vesicles of phosphatidyl choline. The lipid-binding site(s) appears to be located between the amino-terminal myosin head domain and the carboxy-terminal ATP-

insensitive actin-binding domain. Results that are consistent with these will soon be published by Korn's group², who provide several lines of evidence that myosin I, which co-purifies with *Acanthamoeba* plasma membranes, is associated with the membrane in an actin-independent fashion and binds with high affinity to KI-stripped membrane vesicles. A key observation made by these workers is that only the ATP-sensitive actin-binding site on the head domain is accessible to actin when myosin I is membrane-bound. The other site seems to be sterically blocked. Presumably, it is operative when myosin I is off the membrane, where myosin I, like its two-headed cousin, could generate force via interaction between two actin filaments present in the cytoplasm. An attractive notion, discussed by Adams and Pollard¹, is that the association state of myosin I with the membrane may be regulated by phosphatidyl inositol 4,5-bisphosphate metabolism.

It will be critical to establish if these *in vitro* studies are truly reflective of the state of association of myosin I with the membrane *in vivo*. In this regard, immunolocalization studies from Korn's group³ provide strongly supportive evidence that myosin I mediates the membrane-associated cellular movements that persist in the absence of myosin II. Fukui *et al.*³ have simultaneously localized myosin I and myosin II in *Dictyostelium* amoebae and show that myosin I is concentrated at the leading edge of locomoting cells and at sites of phagocytosis, whereas myosin II is found at the cell posterior. Such a distribution is consistent with the unaffected motility-related pseudopod extension and phagocytosis observed in cells with disrupted myosin II. Presumably myosin II could be involved in the retraction of the cell posterior as the cell moves across the substrate — a phenomenon that has been observed for the locomotion of many types of cells.

In addition to their presence in amoeboid cells, two examples of single-headed myosins have also been identified in metazoan species — one in the gut and the other in the eye. In the vertebrate intestinal epithelial cell, the actin filament bundle within each microvillus of the apical brush border is laterally tethered to the

plasma membrane by spirally arranged bridges. In the chicken, these bridges are composed of a large protein complexed to multiple molecules of calmodulin; as reviewed by Louvard⁸, recent studies from several laboratories have shown that this complex is a single-headed, tail-less mechanochemically-active myosin; thus we⁹ have called it brush border myosin I. Sequence analysis¹⁰ of a gene that probably encodes the heavy chain of bovine brush border myosin I reveals that, like amoeboid myosin I, the protein has an amino-terminal myosin head domain and a carboxy-terminal domain without α -helical structure, which has been shown to contain the calmodulin-binding sites of the molecule⁸.

The other single-headed, myosin-like molecule is a product of the *Nina C* gene of *Drosophila*¹¹. This gene encodes a pair of proteins whose expression is required for normal assembly of photoreceptor microvilli in the rhabdoms of the eye. The deduced primary structure of the proteins encoded by *Nina C* indicates that they consist of a central myosin I-like head domain linked to a carboxy-terminal domain unrelated to other myosins. Unlike all other characterized myosins, the proteins have an amino-terminal kinase domain that precedes the head domain. Thus, the *Nina C* locus may encode yet another general class of myosins.

These two examples indicate that there may be widespread expression of single-headed myosins, both of which are membrane associated, in metazoan species. Their structural and functional differences suggest that they could be members of a remarkably diverse family of molecules that include a myosin head as part of their domain structure. It will be interesting to determine how many such 'motors' are expressed in a given species and to compare the properties of the domains that flank the myosin head. Will all single-headed myosins have a carboxy-terminal domain that binds actin and membranes? I suspect we have just seen the iceberg's tip with respect to the functional diversity of this class of myosins. □

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Magnetic fields of spirals

Virginia Trimble

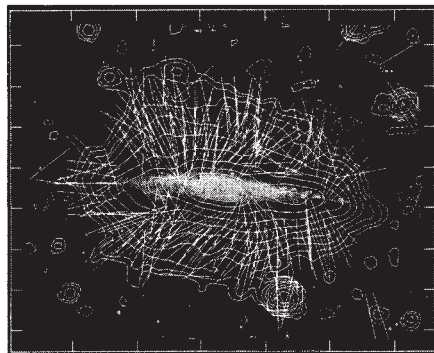
OUR Galaxy had been known to have a large-scale magnetic field for about 20 years¹⁻⁴ when Fritz Zwicky suggested as a hypothesis for its origin: *Dixitque Deus, fiat lux campusque magneticus*^{*}. Another 20 years later, new studies of the face-on spiral galaxy M83 (ref. 5) and of other galaxies discussed at a recent meeting[†] show that we still do not understand galactic magnetic properties well enough to test competing theories of their origin much better than Zwicky could. The competitors are a pre-galactic field, amplified by differential rotation, and dynamos⁶. More of their implications shortly, but it is worth noting immediately that each requires some primordial seed field, though a fairly weak one will do in the dynamo case.

What do real galactic magnetic fields look like? That depends on how you look. Polarization of scattered star light and of synchrotron emission reveal the orientation of the ordered field component, but only in the plane of the sky and to within $\pm 180^\circ$. Faraday rotation of the polarization (with some assumptions about electron density distribution) indicates the strength of ordered fields and the absolute orientation, but again only in the plane of the sky. Thus we probe the field in the disk for spiral galaxies seen face-on and the field perpendicular to the disk for edge-on galaxies.

Most face-on spirals have ordered magnetic fields of a few microgauss (μG) lined up roughly along their spiral arms, according to Marita Krause (Max Planck Inst. for Radioastronomy), Yoshiaki Sofue (Univ. Tokyo) and others. The randomly oriented component is of similar strength. Rainer Beck (Max Planck Inst. for Radioastronomy) noted that, at least in NGC6946, the field direction is definitely that of the spiral arms, not that of the circular gas motion. For two galaxies, M31 (the Andromeda Nebula) and IC342, Faraday rotation data show that the field points the same direction along all the arms, a configuration called an axisymmetric spiral (ASS). In others (M81, M51 and possibly M33), the magnetic field lines change direction by about 180° between arms, forming a bisymmetric spiral (BSS).

In many face-on spirals, the intensity of the ordered field is largest between the arms, not in them. This sounds paradoxical, but seems to mean that the field in the arms has been tangled up by gas turbulence (caused by stellar winds and explosions) so that it is stronger than the inter-

arm field, but less ordered. The study of M83 by Sukumar and Allen, reported on page 537 of this issue⁵, reveals an extreme version of the apparent paradox. The entire inner 12-kiloparsec (kpc) region, with its active star formation and conspicuous spiral arms, shows a largely chaotic field (at 2-kpc resolution). The strongest linearly polarized emission is confined to two 30-kpc arcs, roughly opposite each other in the dark outer



Orientation of the magnetic field lines of NGC4631 projected into the plane of the sky, superimposed on the contour lines of radio emission and an optical photograph. The directions shown are those of B of the 20 cm synchrotron emission, and the lengths are proportional to percentage polarization. (Courtesy of Richard Wielebinski, Max Planck Inst. for Radioastronomy.)

regions of the galaxy, and not associated with optical spiral structure.

In edge-on spirals also, we can see only a projection onto the plane of the sky. Field lines are largely perpendicular to the galactic planes, extending outward for several kiloparsecs. The enraged hedgehog⁷, NGC4631 (see figure), is an extreme case, but most other edge-on galaxies studied show some similar structure. Surprisingly, this perpendicular field is not thought to be a separate (for example, a dipole) component, but rather represents disk field dragged up and out by expanding supernova remnants and other bubbles, columns and fountains of hot gas, according to Eugene N. Parker (Univ. Chicago) and Mitsunori Fujimoto (Nagoya Univ.).

Our Milky Way is normal in having both an ordered field in the plane and spurs extending out of it, first seen via polarization of star light and radio synchrotron emission, respectively. But Faraday rotation of pulsar radiation tells us more, because the dispersion of the pulses measures the intervening electron density separately. Thus we can trace out the strength and orientation of both ordered and chaotic field.

Two analyses, by Richard J. Rand and

Shrinivas R. Kulkarni (California Inst. Technology) and by Andrew G. Lyne and F. Graham Smith⁸ concur at least approximately. In our local spiral arm, a coherent magnetic field of $2-3 \mu\text{G}$ points at roughly 90° from the direction to the Galactic Centre. The chaotic component, on scales less than 100 pc, is, if anything, rather stronger. Alignment of the field with the arm is not firmly excluded, but a ring-like structure is a better fit. The observed Faraday rotation of extragalactic radio sources also favours field lines along gas-streaming directions rather than along the arms⁹. Reversals of the field direction to about 270° occur within 1–2 kpc both inward and outward from our position, and at least one flip back to 90° is probably closer to the centre. Thus the Milky Way cannot readily be described as either an axisymmetric or a bisymmetric spiral.

What do the models of galactic field origin predict? In the case of a pre-galactic ('primordial' or 'praeval') field, caught in conducting interstellar gas and so amplified and stretched out by differential rotation, we expect the field lines, whatever their initial orientation, to lie now largely in the plane, to point in the azimuthal direction, and to reverse their sense up to 100 times along a radius vector through the galactic disk (since spirals are about 100 rotations old). The first two expectations are met by real galaxies; the third distinctly is not. Reversals occur a few times, if at all, from disk centre to edge. But this need not be a fatal objection. When proper account is taken of the diffusion of field lines through the molecular component of the gas and between galactic disk and halo, a typical spiral should display only one or two windings (Russell M. Kulsrud, Princeton Univ.). The resulting configuration will then resemble a bisymmetric spiral.

Dynamos are more complicated. The laboratory sort (the first of which was constructed by Werner von Siemens a bit more than 100 years and a bit less than 100 km away from the Symposium) consists of a conductor rotating through a magnetic field. This generates an electric current in the conductor, which can power an electromagnet to produce the requisite field, which then generates a current and so on, once you get started. Energy is extracted from whatever keeps the conductor rotating. The simplest possible field configuration is a dipole, but you could twist the conductor into any shape you wanted.

In astrophysical contexts, the geometry is necessarily different, owing to the shortage of rigid conducting disks and thin wires. But it is true that there exist patterns of motion in conducting fluids that will regenerate magnetic fields¹⁰ (see also the forthcoming News and Views article by Moffatt¹¹). The flow pattern must include angular velocity that changes

^{*} And God said, let there be light and magnetic field.

[†] IAU Symposium 140 *Galactic and Intergalactic Magnetic Fields*, Heidelberg, 19–23 June 1989.

away from a rotation axis and cyclonic motions — loosely, turbulence with a net twist. And there has to be a bit of field (of almost any geometry) there to start with. The poloidal and toroidal components of the dominant field mode are both axisymmetric. Stars with convective envelopes (like the Sun) and terrestrial planets with metallic fluid cores (like the Earth) meet the required conditions, and their magnetic fields are generally attributed to dynamos.

The disk of a spiral galaxy has the requisite differential rotation, and turbulence is constantly fed in by stellar winds and supernova explosions. Two consequences suggest themselves if galactic fields are dynamo-produced. First, axisymmetric spiral morphology should be favoured, though the dominant mode can be suppressed in several ways, so that an $m = 1$ bisymmetric field is seen. Possible promoters of the BSS configuration include a nearby companion galaxy (Beck), a high ratio of turbulence to differential rotation (Friedrich Krause, Astrophysical Observatory, Potsdam), a relatively thick gas disk (Makoto Tosa and M. Chiba, Tohoku University) and other more subtle gas-dynamic effects (Alexander A. Ruzmaikin, IZMIRAN, Moscow; Yulia S. Krashenninnikova and Anvar M. Shukurov, Space Research Institute, Moscow).

Second, there ought to be some intimate connection between field morphology and the processes responsible for star formation, in and out of spiral density waves. Leon Mestel (Univ. Sussex), A. N. Nelson (Univ. Wales), Fujimoto, Tosa and Chiba all emphasized the synergistic relationship between density waves and dynamos, especially BSS ones.

Galaxies where star formation is primarily driven by density waves are supposed to be the ones with grand designs dominated by two main arms, whereas localized star formation processes give rise to multi-armed and flocculent spirals^{12,13}

Can we say that “all” BSS magnetic fields occur in grand design spirals? Yes, but “all” at the moment means two (M51, M81) or perhaps three (M33) examples. And both the ASS fields (M31 and IC342) occur in multi-armed spirals. But the Milky Way fits into neither category. It has several (perhaps four) major spiral arms and a number of subsidiary ones, but a magnetic field with several reversals. If the field lines follow the gas flow direction rather than the arms, then it is neither ASS nor BSS. Shukurov pointed out that a linear combination of several dynamo modes can produce such a ring-like (axisymmetric, but with reversals) field morphology. Kulsrud’s primordially derived field configuration would seem to be an equally good fit. He also noted that, where the random field component $\langle B^2 \rangle$ is larger than the uniform component $\langle B \rangle^2$ as we find in the solar neighbourhood, then the dynamo equations are not a good approximation anyway.

Perhaps we should not yet feel forced to accept Zwicky’s hypothesis, but it seems at least possible that astronomers are not, at the moment, asking quite the right questions about the nature and origin of galactic magnetic fields. □

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BEHAVIOURAL GENETICS

Nature – nurture and intelligence

Matt McGue

PHILOSOPHERS, poets and scientists have long been intrigued by the relative influence of nature and nurture on human behavioural variability. Plato flirted with eugenics in designing his ideal society, Shakespeare emphasized heredity in character development, Locke and Mill espoused radical environmentalism and Galton devoted much of his illustrious career to a controversy that became synonymous with his name. For the past 50 years the nature–nurture debate has set the research agenda in human behavioural genetics. And, while a balanced review of

behavioural genetic research on intelligence leads to the conclusion that both genes and the environment play an essential role in the development of individual differences in intelligence, as measured by IQ, for some the debate lingers on. The carefully designed French adoption study reported by Capron and Duyme on page 552 of this issue¹, by clearly showing that the IQ of children is influenced by both their biological background and the circumstances of their rearing, should help behavioural scientists move beyond tired controversies and begin to address the real

issues surrounding the mechanisms of genetic and environmental influence.

Genetics provide the behavioural scientist with a powerful set of tools for unravelling the sources of individual differences. The simple elegance of the Capron and Duyme design, lost perhaps on those accustomed to precise experimental control, is rarely seen in non-experimental behavioural research. They have no need for the obfuscating statistical models and attendant unsupportable assumptions that burden so much similar research. The effects Capron and Duyme report are strong and indisputable. Their study clearly illustrates the utility of behavioural genetic methods and highlights the issues that need to be addressed in future research. Four issues are relevant.

Environmental influence

Capron and Duyme report that the average IQ of French adoptees is some 12 points higher when they are reared by parents with high rather than low socioeconomic status (SES). This is a sizeable effect and approaches the difference between the mean IQ of students admitted to US colleges and that of the general population. At first this may seem a trivial observation because high-SES parents have children who perform well on IQ tests. But it is not. By demonstrating a relationship between characteristics of adoptive parents and the test performance of their non-genetically related offspring, Capron and Duyme necessarily implicate an environmental effect. There have been numerous studies relating parental SES to the IQ of their offspring in intact biologically related families. These studies, however, confound genetic and environmental sources of familial resemblance. Failure to control for genetic sources of resemblance has often led to erroneous inferences about parental environmental influence (for example, the schizophrenogenic mother). One of the greatest challenges to behavioural genetic research is the need to control for genetic factors when investigating familial environmental influences. The adoption study provides such control.

Although Capron and Duyme provide unequivocal evidence for the existence of an environmental influence, they do not identify the mechanism of that influence. Parental education and SES are ambiguous indicators of the intellectual environment of the child. Working class parents can provide their children with intellectually stimulating experiences and professional parents can neglect the intellectual needs of their children. It remains unclear whether the SES effect is related to access to quality education, the variety and complexity of intellectual stimulation in the home, the parents’ press for scholastic achievement, or some other factor that differentiates between high- and low-SES homes². At least one adoption study has

shown that maternal encouragement is related to adopted children's degree of intellectual confidence which in turn is related to cognitive test performance³. What are needed now are more adoption studies aimed at determining not whether, but how, parents contribute to the intellectual environment of their children.

Genetic influence

Capron and Duyme report that adoptees with high-SES biological parents have a mean IQ approximately 15 points higher than adoptees with low-SES biological parents — another sizeable effect. Biological background may be related to pre-natal teratogenic exposures, peri-natal complications and poor pre-placement history, all of which might affect adoptee IQ. Given what we know about the magnitude of the effect of these factors, however, it is unlikely that they alone could account for the entire observed difference. A sizeable portion of the 15-point mean IQ difference can safely be attributed to genetic influences, which is wholly consistent with other twin, adoption and family studies of IQ⁴. Strangely, Capron and Duyme carefully avoid a genetic interpretation of their results and thereby fail to interpret their findings within the broader research context.

Two alternative hypotheses have been advanced to account for genetic influences on IQ. The first might be termed the neural efficiency hypothesis. High-IQ individuals are characterized by an ability to process information rapidly and efficiently⁵. A given cognitive task will demand fewer of the information-processing resources available to an individual with high IQ than to somebody with low IQ, freeing the individual to engage in the higher-level analytical processing that is the hallmark of intelligent behaviour. Information processing has been shown to be under partial genetic influence in both mice and humans⁶, supporting the view that a genetic effect upon the structures and systems of the central nervous system might comprise the biological substrate of intelligence. Recent advances in neuro-imaging that allow increased precision in assessment of anatomical structure and brain physiology should greatly aid in the task of identifying the mechanisms of genetic influence on IQ.

An entirely different explanation might be termed the environmental mediation hypothesis⁷. Consider a simple vocabulary test. Vocabulary is featured prominently in most omnibus tests of intelligence, is highly related to other cognitive abilities and is nearly as heritable as IQ. Does the genetic influence on vocabulary reflect equal opportunity to learn the meaning of words with only those with the most efficient nervous systems able to take full advantage of that opportunity, or marked differences in the occasions for learning?

In all likelihood it is a little of both. Individuals play a significant role in determining their experiences, learning or otherwise. The student who excels in exams and always turns in homework on time is likely to elicit a very different set of reactions from teachers and parents than is a low-achieving, conduct-disordered child. A competent and confident child is more likely, when given the opportunity, to select a challenging course of study than is a child who has struggled academically. And so on. Once one grants that behaviour drives experience, it is a small leap to allow that genetic factors might influence our environmental circumstances. As my colleague D. T. Lykken puts it, the issue properly framed is not so much nature *versus* nurture as it is nature *via* nurture.

Interactions

Possibly the most provocative and novel finding from the French adoption study is the absence of an interaction between biological background and rearing circumstance. The mean IQ of adoptees is approximately 12 points higher when they are reared in a high rather than low-SES home, irrespective of biological background. Geneticists have long recognized the importance of genotype-environment interactions. Nonetheless, there is very little empirical support for their existence in the behavioural domain — a state of affairs lamented by at least one prominent behavioural geneticist⁸. We should not go so far as to conclude, however, that there are no inherited differences in sensitivity to environmental differences. The environmental difference in the Capron and Duyme study is within the normal range of rearing circumstances and the biological background of the adoptees falls within the normal range of parental status. Perhaps interactions should only be expected between the extremes of biological background and/or environmental circumstance: it is unlikely that any but the most musically gifted child would have benefited at so early an age from the intense training provided to Mozart by his father. At the other end of the continuum, children of average ability would not derive the same benefits from basic skill training as would children with severe mental defects. In all likelihood, significant interactions do exist; we just need to know where to look for them.

Developmental course

Most behavioural genetic research on intellectual ability has adopted a static perspective. IQ, although highly stable over short periods of time, changes during development. A genetic or environmental influence at one point in time may not operate at another. The adoptees in the Capron and Duyme study had an average age of 14. Will the magnitude of the biological and environmental effects dimin-

ish, remain stable or increase after these children reach maturity and leave home? Unfortunately, little research addresses this question. Although it is easy to imagine genetic factors having their maximal influence early in life and declining thereafter as individuals are exposed to greater and more varied experiences, the few twin and adoption data that exist suggest that the relative importance of the genetic influence on IQ increases at least until late adolescence⁹. On the other hand, it could be argued that the influence of the rearing family should be greatest at the time when cognitive abilities are rapidly developing and when the children are still residing in the home. This proposition has received some empirical support. Studies of adoptees in late adolescence and early adulthood suggest a reduced impact of rearing SES as the child matures¹⁰. It will be important to follow the French sample, as well as other adoptee samples, into adulthood to determine the long-term effects of rearing circumstance and biological background.

Much of the interest, indeed the contentiousness, of the nature-nurture debate stems from the supposed social policy implications of behavioural genetic research on IQ. The whole basis for the controversy becomes confused, however, once it is realized that environmental effects can include irreversible pre-natal traumas and that genetic effects can include the environments we create for ourselves. Much nature-nurture research on IQ has focused on describing what *is*, rather than inferring what might be. In the absence of an understanding of underlying mechanisms, any implications of the research for targeting interventions (and there may well be none) are at best uncertain.

Most scientists are now ready to accept the existence of genetic influences on behavioural variability. This acceptance has at once signalled a victory for behavioural genetic researchers and served them a challenge to re-establish the vitality of their discipline. The day has arrived when showing that some trait is under partial genetic or environmental control without accounting for, or hypothesizing about, the mechanism of that influence will simply not do. □

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Growth of lithospheric mantle

Shen-su Sun

CHEMICAL differentiation of the Earth into the core, mantle and crust is a result of thermal evolution involving melting and density stratification. The lithospheric mantle in both oceanic and continental environments can be considered as a cool conductive layer attached to the crust and overlying the convective asthenospheric mantle. The thickness of the lithosphere depends on its age and tectonic environment, ranging from less than 120 km for the oceanic lithosphere to as much as 200–250 km for the stable continental cratons. The common view of the growth of continental lithospheric mantle was challenged last year by Salters and Shimizu¹ who suggested that both continental and oceanic lithospheric mantle include a significant component derived from material that has undergone elemental fractionation by melting in the deep mantle. It is challenged again on page 548 of this issue² by Jochum *et al.* who suggest instead that growth occurs mainly by underplating by asthenospheric material with a composition similar to that of sources of mid-ocean-ridge basalts and residues from ocean-island basalts.

A clear understanding of chemical composition and modes of formation of the lithospheric mantle is important to issues such as growth and evolution of the continental crust, the fate of the subducted oceanic lithosphere and mantle dynamics. It is also relevant to the petrogenesis of basalts occurring at the mid-oceanic ridge

(MORB), ocean islands (OIB) and island arcs (IAB), the origin of diamond in the lithospheric mantle and the chemical and isotope budgets of the mantle–crust system.

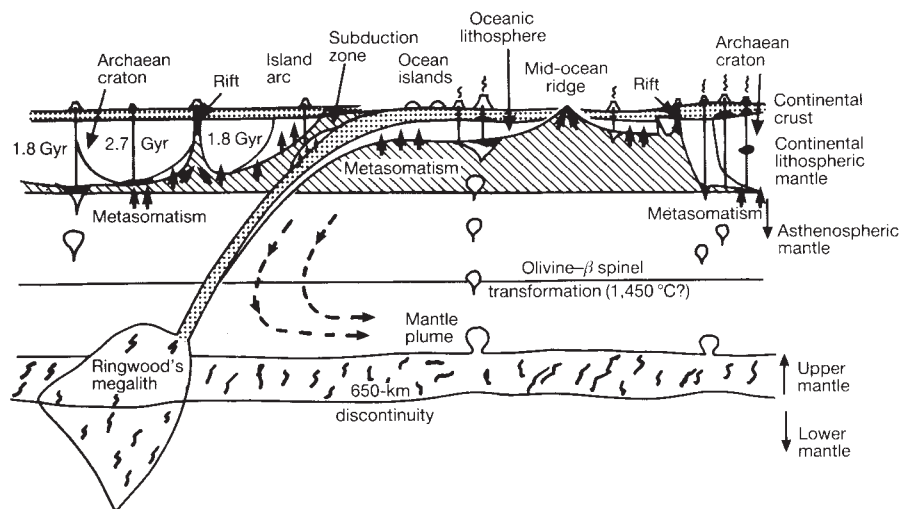
The common view is that continental lithosphere forms via many physical processes; in contrast, oceanic lithosphere simply thickens as the lithosphere cools and migrates away from a spreading centre (see figure). The stabilization of the earliest continental crust occurred during the Archaean (4.0–2.5 × 10⁹ years ago) and was accompanied by the formation of a thick, harzburgitic lithospheric mantle up to 200 km thick. Such old continental lithosphere may also contain eclogite bodies which represent trapped basaltic melts and subducted oceanic crust. Subsequent evolution occurred in diverse ways. Fertile or other refractory mantle components may be accreted either through cooling or from mantle plume activities. Multiple crustal accretions to the Archaean cratons result in the modification of existing continental lithosphere and the formation of new lithosphere by intrusion and underplating. The lithosphere may be thickened by continental collision or downward advection. On rare occasions, delamination of some parts of the lithosphere and their return into the convecting asthenosphere may be induced by extensive magma intrusion or a continental collision.

In subduction-zone environments, fluids

and melts derived from the mantle wedge and by dehydration and partial melting of the sinking oceanic crust can rise and so modify continental lithospheric mantle. Subducted oceanic lithosphere (under favourable circumstances) and domains of refractory peridotite within the mantle wedge also may be involved in growth of the continental lithosphere during or after subduction. Continental lithosphere in a rifting environment is continuously stretched and thermally thinned; upwelling of asthenospheric mantle progressively replaces existing older lithosphere. Subsequent decay of such thermal anomalies allows for further growth of the continental lithosphere. The combined outcome of these diverse processes is a chemically and isotopically heterogeneous continental lithospheric mantle^{3,4}.

Both Jochum *et al.*² and Salters and Shimizu¹ challenge this view, although in incompatible ways. Jochum *et al.* use spark-source mass spectrometry and radiochemical neutron activation to analyse trace elements in five anhydrous spinel lherzolite samples, four from continental rifts and one from the Massif Central in France. The depletion pattern for Th, Nb, Ta, Sr, Zr, Hf and rare-earth elements in these peridotite samples (their Fig. 3 on page 550) is the same as that expected for sources of MORB and residues from intraplate basalts such as OIB. The authors suggest that continental lithospheric mantle grows mainly by underplating of refractory diapirs produced in a divergent margin (such as continental rift) or in an intraplate tectonic setting (over a mantle plume). They found no evidence of subduction-zone modification.

Salters and Shimizu used an ion microprobe to analyse clinopyroxenes in peridotite rocks from continental, oceanic and island-arc environments. They also attempted to show that the clinopyroxenes, the main hosts of certain elements in anhydrous peridotites, are representative of the whole rocks. Their data, when normalized in the same way as in Fig. 3 of Jochum *et al.*, show that most samples are strongly depleted in Zr and Ti relative to the rare-earth elements and so cannot be related to MORB or OIB either as a source or as a residue. Although the Zr- and Ti-depleted character of the clinopyroxene samples is similar to some calc-alkaline volcanics of island arcs, they consider that this depletion character was not generated in the mantle wedge above the subduction zone. Otherwise a mechanism, as yet obscure, is required to entrain and transport this mantle material to high levels in the oceanic mantle without affecting the mantle sources of MORB and OIB. They therefore speculate that high-pressure phases in the deeper part of the upper mantle and the lower mantle (such as garnet, majorite and perovskite) could fractionate Ti and Zr from the rare



The diagram illustrates formation of lithospheric mantle and generation of mantle heterogeneity through plate tectonic processes, including lithosphere subduction, upward migration of silica-undersaturated melts from the asthenosphere, and growth of continental lithosphere through underplating of mantle-derived material, at different times and different tectonic settings. To the left, lithospheric mantle of the late Archaean (2.7 Gyr) craton is shown to be surrounded by lithospheric mantle formed during early Proterozoic (1.8 Gyr) and younger ages. Different components involved in magma generation in different tectonic environments are also shown. Differences of opinions on the issues of whole- or layered-mantle convection and the exact forms of mantle plumes and Ringwood's megalith do not affect the discussion presented here. (Modified from Fig. 7.7.1 of ref. 4.)

earths and so play a role in creating this depleted character through melting. The subsequent rise of such refractory and lighter mantle material to low-pressure environments contributes to the growth of much of the lithospheric mantle. This mantle, the authors suggest, could become the source of calc-alkaline volcanics in the subduction zones when its solidus is lowered by flux of volatile elements released from the subducted oceanic lithosphere.

These ideas not only challenge the current popular views on lithospheric mantle; they are also mutually incompatible. Some serious questions need to be asked. The different approaches could have their own merits and inherent shortcomings so that each reveals only parts of the story. Can the samples studied be considered as truly representative of the lithospheric mantle? Is each interpretation the unique one for the data and are the proposed models internally consistent? What happens to the mantle residue after generating MORB at high levels of the mantle? Why is it not involved in formation of the oceanic lithosphere to become a source of island arc basalts? What are the physical and chemical consequences of melting in the deep mantle involving majorite and perovskite?

Note that Jochum *et al.* show that in their peridotite sample D1, Ti, Zr and Hf are not depleted relative to rare-earth elements, but that its clinopyroxene separate is strongly depleted in these elements. Therefore Ti, Zr and rare-earth abundance pattern of the clinopyroxene may not necessarily represent the whole rock, contrary to Salters and Shimizu's notion. Furthermore, the different relative depletions of these elements can be achieved by metasomatic enrichment in intraplate environments as well as at subduction zones. Metasomatism of the lithospheric mantle is a natural consequence of upward migration of melt and fluid in the upper-mantle melting zone (shaded region in the

figure). McDonough and I noted⁵ that depletion of Ti, Zr and Hf relative to rare earths is common for kimberlites, carbonatites and some ocean-island basalts with high levels of light rare earths — that is, for magmas generated by very small degrees of melting. Nephelinites of Oahu island are good examples⁶. We proposed that this depletion could be related to many factors: changes in the residual-mineral composition (such as the appearance of titanates or an increase of garnet abundance with depth); mineral/melt partition coefficients; and magma-generation processes (for example, zone refining becomes important). Melting of the recycled oceanic crust in the form of entrained, stretched veins of eclogites in the upper-mantle sources of MORB and OIB may also be important.

Further chemical and isotope data are clearly needed, especially for peridotite samples from various tectonic environments and with light rare-earth enrichment. Studies of xenoliths and massifs as well as studies of mafic-ultramafic magmas demonstrably derived from melting of the metasomatized continental lithospheric mantle should help clarify which, if either, model describes lithospheric growth best. Although the new papers should be assessed with caution, it is apparent that they will stimulate important new approaches in the subject. □

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DEVELOPMENT

An eye to the main chance

Andrew Tomlinson

NEW systems in which to investigate the molecular basis by which organisms make developmental decisions and then execute the genetic programmes that lead to the production of different cell types are always welcome. One that has recently emerged is the compound eye of the fruit fly, *Drosophila*. On page 531 of this issue¹, Moses *et al.* report that the *glass* gene of *Drosophila* is essential for the correct development of the photoreceptor cells in the eye (and elsewhere), and that the gene encodes a protein of the 'zinc-finger' type, other examples of which have previously

been identified as important in developmental processes. This information on *glass*, together with recent information on two other genes, *sevenless* and *rough*, confirm the growing view that the *Drosophila* eye is a particularly advantageous system for developmental biologists to study.

Drosophila's compound eye is made from many hundred ommatidia, each of which is a simple array of photoreceptors and accessory cells arranged in a precise spatial array. Ommatidia are constructed in a monolayer epithelium allowing individual cells to be identified and followed

as development occurs. The cells are directed to their fate by signals passed from neighbouring cells, and genetic screens can be used to identify flies with mutations in these communication mechanisms and the differentiation processes they trigger. Once a mutant is isolated, genetic manipulations can be used to establish whether the mutation acts in the signalling mechanism or in the reception, interpretation and differentiation process.

The *glass* gene gets its name from a mutation, discovered by Muller in 1918, that causes a roughening of the compound eye. The first indication of the role of *glass* came when Richard Lebovitz, working in Donald Ready's laboratory, found that the developing photoreceptors in *glass* mutants begin to differentiate normally but fail to progress to the expression of photoreceptor-specific protein and later die (see ref. 2). These data encouraged the present analysis, in which Moses *et al.* show that *glass* gene function is required in the photoreceptors, which die without it, but in no other cell type of the eye. As the photoreceptors show signs of early differentiation but then do not progress, the protein encoded by *glass* is seen as controlling the differentiation of the photoreceptors.

Like several other proteins implicated in developmental mechanisms in *Drosophila*^{3–5}, the *glass* gene product contains a zinc finger — an amino-acid motif that predicts the existence of a zinc-chelating finger-like structure that binds to DNA and acts as a regulator of gene transcription.

The product of *sevenless*, by contrast, is a transmembrane protein with a large extracellular domain and an internal tyrosine kinase, and is similar in structure to peptide hormone receptors⁶. Removal of *sevenless* function causes the cell normally destined to form the ultraviolet photoreceptor, R7, to differentiate into a non-photoreceptor. Although supplied with the correct developmental signals, the progenitor of R7 is unable either to receive or to interpret them⁷. Thus, *sevenless* is probably a protein that transduces positional signals directing the R7 developmental pathway by a process in which binding of a signalling ligand to the protein's extracellular domain modulates its internal tyrosine kinase activity. (The function in the *Drosophila* eye of another probable transmembrane receptor with tyrosine kinase activity — the fly's equivalent of the mammalian epidermal growth factor receptor — is not clear, but it may be involved in longer range interactions than *sevenless*⁸.)

A third gene of interest in eye development is *rough*, which is required in photoreceptors R2 and R5 for inductive signals to be sent to photoreceptors R3 and R4. The gene encodes a protein containing a homoeodomain⁹. Like zinc fingers, homoeodomains are DNA-binding and

are involved in transcriptional regulation. Many developmentally important genes in *Drosophila* and other species encompassing many phyla, have been found to encode homoeodomains. The interesting feature of the *rough* protein is that it appears to control, at the transcriptional level, the expression of signals from one cell type that are received and interpreted by others.

By comparison with research on *Drosophila* embryos, eye development has been less intensely studied. But it has two distinct features in its favour. First, the pattern formation is more simple and easy to follow than in the embryo, and less complex developmental questions that focus on the behaviour of single cells can be asked. Second, the entire visual system can be removed without affecting the viability of the fly, and extensive manipulations of the system are possible. This latter point becomes even more important when it is realized that flies are still viable in the absence of function of *glass*, *sevenless* and *rough*. This will allow extensive

analyses of the various protein functions to be performed in a way that is not possible for lethally mutant genes. For example, it will be possible to modify any of the three genes *in vitro*, reintroduce them into a mutant fly and then assay the effect on eye development. In this way the various operational domains of the proteins can be biochemically dissected in a systematic way and further insights into their function gained. □

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CELL DIVISION

Photoactive microtubule flux

Roy Burns

THE mitotic spindle, a complex structure that organizes the separation of the chromosomes during cell division, consists of two kinds of microtubule fibres. The pole-to-pole microtubules slide relative to each other in an antiparallel fashion to effect anaphase B (see figure). The kinetochore microtubules link the chromosomes to the poles of the spindle. In a new paper¹ in the *Journal of Cell Biology*, Tim Mitchison reports a novel and elegant approach to demonstrate the flux, *in vivo*, of tubulin subunits along the kinetochore microtubules during metaphase, which complements earlier work showing that these microtubules shorten during anaphase A.

Mitchison has turned on its head the widely used technique of fluorescence recovery after photobleaching. He has prepared a 'caged' derivative of the molecule FITC, which becomes fluorescent only on photoactivation, and coupled it to tubulin. This complex becomes incorporated into the spindle microtubules upon microinjection into cells at metaphase, where the chromosomes are at the equator of the spindle. Using light at a wavelength of 365 nm, Mitchison irradiated a narrow bar across the spindle between the chromosomes and the poles; the bar fluoresces at a longer wavelength and its fate can be monitored as the cells move through metaphase to anaphase.

Mitchison observed an initial rapid decrease in the intensity of the photo-

activated bar and a general increase in the background fluorescence, which he ascribes to the turnover of the more labile pole-to-pole microtubules. The residual fluorescence of the bar appeared to be restricted to the bundles of kinetochore microtubules, and this fluorescence slowly migrated towards the spindle poles while the cells remained at metaphase. As the chromosome-to-pole distance remained approximately constant, this movement of the residual fluorescence clearly demonstrates continued addition of tubulin subunits at the kinetochores and loss of subunits at the poles.

This flux resembles the treadmilling, or head-to-tail polymerization, of microtubules assembled *in vitro* to steady state^{2–4}, and which results from a difference in the critical concentration at the two ends of the microtubule (see figure). Although the observed flux is in the direction predicted from the polarity of the microtubules, Mitchison notes that treadmilling is only one possible mechanism, as it could also be effected by one of the various mechanochemical motors (see ref. 5).

It has long been unclear whether treadmilling is of any relevance *in vivo*, as most microtubules terminate at their slow-growing or minus ends in specialized structures called microtubule-organizing centres, such as the centrosomes. Indeed, the kinetic difference between the two ends has been postulated to be a mechanism for restricting microtubule assembly

to such organizing centres⁵, so establishing the specific spatial organization characteristic of the microtubule cytoskeleton. The close association of the minus ends with such structures might be expected to prevent subunit addition and loss: treadmilling would simply be an artefact resulting from the availability of the two ends *in vitro*.

This argument against treadmilling occurring *in vivo* has looked rather flimsy since Mitchison and collaborators demonstrated, using *in vitro* microtubule-chromosome constructs, that microtubules could assemble⁷ or disassemble⁸ at the kinetochore-microtubule junction without transitory detachment. However, both ends of the microtubule must be kinetically active if subunit flux is to occur: Mitchison's new studies¹ show that this condition is satisfied, at least for the kinetochore microtubules during metaphase.

Although treadmilling has been considered in terms of subunit flux along the microtubule, the evidence has relied heavily upon the kinetics of incorporation of radiolabelled GTP in pulse-chase experiments. Interpretation of these kinetics was complicated by the subsequent demonstration that microtubules can exhibit dynamic instability (see ref. 9) resulting from the simultaneous elongation and shortening of distinct subpopulations. Both behaviours require microtubules to respond to two different critical concentrations, either at the two ends or between the microtubule subpopulations, and at first sight the GTP data could be interpreted by either alternative.

Further detailed studies, however, confirm that treadmilling and dynamic instability are not mutually exclusive, and that the presence or absence of microtubule-associated proteins can push

100 years ago



DO CATS COUNT?

ABOUT two weeks ago, the cat of a dairyman in this neighbourhood gave birth to three kittens. Next day, one of them was removed, during the mother's absence, and drowned. On returning from a foraging expedition, and discovering her loss, puss immediately set out in search of the missing one. All her efforts in this direction, of course, proved fruitless; but, evidently determined to at least make up the right number, she did so by carrying off, from its nest close by, a young hare, not more than a week old. This she is at present suckling side by side with her own kittens. In view of these facts the above question very naturally suggests itself.

J.T. WALKER

From *Nature* **40**, 385; 22 August 1889.

a-c, Metaphase spindle showing the pole-to-pole and kinetochore-to-pole microtubules, and illustrating the flux of tubulin subunits (black circles) in the kinetochore microtubules. a, Mitchison¹ has irradiated a bar across the spindle, close to the chromosomes, to photoactivate modified tubulin subunits microinjected into the cells and incorporated into all the spindle microtubules. The photoactivated subunits can then be seen by fluorescence microscopy. b,c, Such subunits in the highly labile pole-to-pole microtubules are rapidly and uniformly dispersed throughout the spindle, and give rise to a low background fluorescence. By contrast, the photoactivated subunits of the kinetochore microtubules flux at constant rate towards the spindle pole. d, Treadmilling in which hypothetically labelled subunits migrate with time from the net assembly (A-) end towards the net disassembly (D-) end⁴. The A-end has a lower critical concentration, that is, the ratio of the dissociation to the association rate constant or the concentration of tubulin subunits remaining at steady state. The treadmilling is shown occurring from the fast-growing (+) end towards the slow-growing (-) end. e, Comparison of the kinetic properties of kinetochore microtubules during metaphase and anaphase A, showing the (+) end associated with the kinetochore and the (-) end with the spindle pole. During metaphase, there is net subunit addition at the kinetochore and loss at the pole, leading to subunit flux¹. f, By contrast, there is net subunit loss from the kinetochore during anaphase A, resulting in the chromosome moving towards the pole⁸.

the *in vitro* system towards one or other behaviour¹⁰. Furthermore, direct visualization of individual microtubules shows that the length excursions characteristic of dynamic instability differ at the two microtubule ends^{11,12}, consistent with the basic requirement of treadmilling for the two ends to be kinetically different. Similarly, when microtubules grown off the two ends of isolated axonemes are cut by laser irradiation, the portion still attached to the axonemal plus end rapidly disassembles, whereas that remaining attached to the minus end is remarkably stable¹³.

Treadmilling is, therefore, a genuine property of microtubules assembled *in vitro* to steady state, and key questions now are whether it drives the observed flux *in vivo*; whether it is a process for effecting work¹⁴; how the activity of the plus and minus ends are controlled *in vivo*; and how the cellular flux relates to the observed dynamic instability of individual microtubules¹⁵⁻¹⁷. Although the relationship between treadmilling and dynamic instability is tractable *in vitro*, resolving the exact cellular behaviour of specific microtubules is a far more challenging problem. Indeed, the cellular behaviour of a specific microtubule may not only change with time, but it could also involve regulatory factors which have yet to be identified.

This is amply illustrated by the behaviour of the kinetochore microtubules, which flux during metaphase but which shorten during anaphase A (see figure).

Microtubule disassembly at the kinetochores, which mirrors the process of anaphase A (see my News and Views article last year¹⁸), can be induced by lowering the exogenous tubulin concentration⁵. By contrast, flux involves subunit addition at the same site and needs both a higher exogenous tubulin concentration and ATP⁷. The ATP requirement suggests that the kinetochore contains a mechanochemical ATPase or a protein kinase, so that the switch between metaphase and anaphase A is likely to involve a control of the effective tubulin concentration and kinetochore-associated enzyme(s). □

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Radiant rubbish

THE ideal way of getting rid of urban and industrial waste is to detoxify and reclaim it. Daedalus has been studying nature's own methods of detoxification (such as oxidation in the liver) and reclamation (digestion, essentially reaction with water). So he is inventing a hydrolytic-oxidation rubbish-disposal process.

The biological components of rubbish, like waste food, paper, wood and natural fibres, will all readily hydrolyse, mainly to sugars and amino acids. Condensation polymers should also hydrolyse, back to alcohols and organic acids. Other plastics and organic materials should be vulnerable to hydrolytic oxidation, yielding aldehydes or other oxygenates. Nearly all these products are organic liquids, or solids which should dissolve in them. So the hydrolysis and oxidation of rubbish should produce a convenient 'junk fluid', ideal as a fuel and rich with possibilities as a chemical feedstock.

The snag, of course, is that the comprehensive hydrolytic oxidation of all molecular comers will require some pretty fierce chemistry. At first Daedalus planned to borrow the digestive secrets of such omnivorous creatures as termites, goats and ostriches. But he now aims to enlist the aid of the most ferocious rubbish of all — nuclear waste.

High-energy radiation, of course, breaks chemical bonds indiscriminately, generating copious and highly reactive free radicals. No organic substance can withstand it. In the presence of air and water, hydroxyl and hydroperoxide radicals are produced as well: irresistible agents of oxidation and hydrolysis. At the moment, high-level nuclear waste is entombed in thick concrete shielding which absorbs its radiation while it decays to safer levels. Daedalus proposes instead to surround it with rubbish saturated with air and water. The rubbish shielding will absorb the radiation, and the resulting free-radical oxidation and hydrolysis will rapidly break it down to junk fluid. As fast as it softens and liquefies, more will be added on top to keep the process going and the waste safely shielded. Limpid, clean, and absolutely sterile, junk fluid will trickle continuously out of the base of the reaction vessel. Unreactive components of the rubbish, such as metals and ceramics, would also sink continuously to its base for extraction, but might best be separated in advance.

Thus the noisome detritus of our civilization will be simply and continuously converted into a cheap and useful liquid; and nuclear waste will at last be put to good use. If the waste is well contained, junk fluid will not pick up appreciable radioactivity itself. The chemical industry will happily convert it into solvents, motor fuels and, of course, more plastics. David Jones

Indirect effects of an oil spill

SIR—We were monitoring the population of south polar skuas (*Catharacta maccormicki*) near the US Antarctic research station at Palmer Station when the Argentine Navy ship *Bahia Paraíso* ran aground on 29 January 1989 (see ref. 1). The wreck spilled one million litres of diesel and jet fuel near large seabird colonies (more than 30,000 birds; W.R. Fraser, personal communication) at the height of the breeding season. During the spill, diving birds (penguins and shags) died from fouling and toxicity, and there was an unprecedented^{2,3} reproductive failure in south polar skuas, in which all young died within a 3-week period during the spill. Skua nestlings had normal growth rates during the spill, showing no evidence of infection, fouling or toxicity^{4,5}, such as the haemorrhagic gastroenteritis seen in shag nestlings. We suggest that fouling disrupted the normal parental behaviour of south polar skuas and indirectly caused the reproductive failure.

Whereas the skuas frequently foraged in oil slicks and acquired a light diesel coating, we did not see oiled skuas in their territories. Our observations of a captive bird suggest that skuas require two days of bathing to clean themselves, substantially longer than their normal shift away from the nest⁶. Lapses in nest attendance by adults became common during the spill (frequency of occurrence: 3% before, 31% during, $\chi^2 P < 0.01$) and left young vulnerable to predation by other skuas². Although not observed before the spill, predation was the only known cause of death during the spill and coincided with lapses in nest attendance. Adult pairs were commonly seen defending their territories after losing their young.

Our hypothesis of the cause of the reproductive failure is strongly supported by comparison of south polar skuas with other sympatric species. Reproductive failure was not seen either in the non-cannibalistic species that encountered oil (Adelie penguins *Pygoscelis adeliae*, blue-eyed shags *Phalacrocorax atriceps*, southern giant petrels, *Macronectes giganteus*), or in cannibalistic species that were not oiled (brown skuas, *Catharacta lonnbergii*). Like south polar skuas, brown skuas are opportunistic cannibals and are fiercely territorial. But unlike south polar skuas, which feed mainly at sea, brown skuas feed in penguin colonies², and hence are less likely to become oiled. While south polar skuas suffered reproductive failure during the spill, brown skuas apparently had normal reproductive success.

The short-term effects of the *Bahia Paraíso* spill on this and other populations of seabirds at Palmer Station were unexpected. Instead of minor negative

effects on all seabird species, we saw a range of short-term effects varying from catastrophically negative to neutral and, perhaps, positive — the giant petrel population has produced 30% more young this year (W.R. Fraser, personal communication) in part, perhaps, because of the abundance of food after the spill. Direct toxicity and fouling caused mortality in penguins and shags, the two species which neither foraged in oil slicks nor ate contaminated prey, but which became fouled when diving through oil. These deaths provided abundant food for avian scavengers. By satiating scavengers, which also prey on the fledglings of other seabirds, the oil spill may have conserved productivity in some species.

In a small spill, where the direct effects of oil are not uniformly catastrophic, indirect effects become apparent. The events at Palmer Station emphasize the importance of behavioural and ecological interactions in determining the immediate effects of oil on species. Although these factors may be understood *post hoc*, correctly predicting the effects of oil on species may be difficult.

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Deuterium on Venus

SIR—Bertaux and Clarke¹ place an upper limit of 300 rayleighs (2σ) on the deuterium Lyman- α emission from the disk of Venus, viewed near maximum elongation by the International Ultraviolet Explorer (IUE). They then deduce the column density of deuterium in the upper atmosphere of Venus above 110 km and construct models for deuterium with this columnar abundance to compare with one of hydrogen, obtained from a radiative transfer analysis of Pioneer Venus short ultraviolet data². They conclude that the upper limit to the D/H ratio at 100 km is $(3.6 \pm 1.5) \times 10^{-3}$ and claim that this conflicts with an interpretation of ion-composition data that identifies the mass-2 ion in the night-time ionosphere as D^+ rather than H_2^+ (refs 3–5). They also argue that this D/H ratio may also hold in the bulk atmosphere, rather than the ratio of

$(1.6 \pm 0.2) \times 10^{-2}$ obtained by mass spectrometry⁶.

In view of the compelling reasons for taking the main mass-2 species in the upper atmosphere to be D , rather than H_2 (refs 3–5), and the agreement between the D/H ratio measured for Venus cloud drops⁶ and that implied by the ionospheric analysis^{3–5}, it seems prudent to try to reconcile the IUE results with the large isotope ratio. I believe that the IUE data could concern the aeronomy of hydrogen in the upper atmosphere of Venus, rather than contradicting the evidence for a large D/H ratio.

There is an unacceptable inconsistency in using the hydrogen distribution constructed for the short ultraviolet radiative transport analysis on the one hand and interpreting the mass-2 species in the upper atmosphere as H_2 on the other. The atomic hydrogen distribution in the Paxton model² is calculated on the assumption that there is no H_2 in the upper atmosphere. If there are 10 p.p.m. of H_2 at the homopause (in contrast with 0.7 p.p.m. of H), as Bertaux and Clarke suggest, the aeronomy of hydrogen on Venus will be drastically different from that on Earth⁷, contrary to their assertion. The atomic hydrogen distribution will be nothing like the one used by Paxton *et al.*, from which Bertaux and Clarke took the density at 110 km (Fig. 1). The conversion of H_2 to H will place relatively much more atomic hydrogen at high altitude, where the temperature is higher than it is at 110 km. As Paxton *et al.* emphasize, temperature-dependent scattering effects can change Ly- α emission rates by a factor as large as 2.

In view of the results of Bertaux and Clarke, it is necessary to revisit the radiative transfer calculations to determine whether the short ultraviolet results can be interpreted in terms of a hydrogen distribution in which the ratio of high- to low-altitude (and temperature) hydrogen is increased significantly. Thus, less columnar atomic hydrogen may produce the same nadir Ly- α emission rate, the

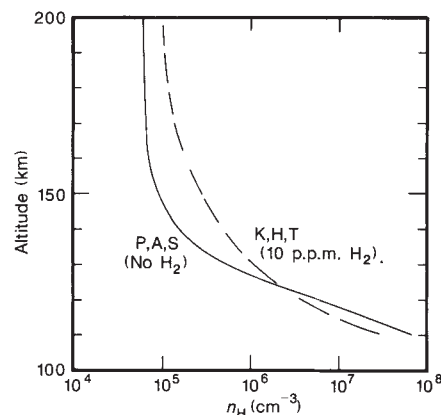


FIG. 1 Atomic hydrogen density with no H_2 at 110 km (ref. 2) and with 10 p.p.m. of H_2 at 110 km (ref. 7).

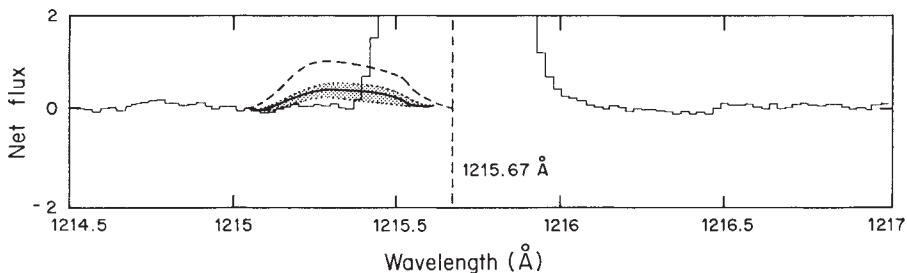


FIG. 2 Observed IUE spectrum near 1,216 Å with 2.5 kR of D Ly- α (dashed line) and 1.0 ± 0.4 kR (stippled region).

optical depth of the corresponding deuterium distribution being reduced. A significant concentration of hydrogen in the form of compounds (such as H_2 and HCl) at 110 km could cause such a change in the hydrogen altitude profile. Although ionospheric physics requires the mass-2 species to be mostly D and excludes 10 p.p.m. of H_2 at 100 km, it may allow 1 or 2 p.p.m. of H_2 .

Note that the figure in the Bertaux and Clarke letter is misleading and its caption is incorrect in asserting that a D/H ratio of 1.6×10^{-2} would lead to 2.5 kR of D Ly- α . The authors mistakenly take the upward flux of hydrogen in Paxton's analysis ($7.5 \times 10^7 \text{ cm}^{-2} \text{ s}^{-1}$) to be escape flux. Almost all this upward flux goes to support global circulation. Hence it is appropriate to treat deuterium in a one-dimensional model as carrying an analogous flux. Proper calculation of the deuterium column density gives $(3 \pm 1.2) \times 10^{11} \text{ cm}^{-2}$ when the D/H ratio at 100 km is $(1.6 \pm 0.2) \times 10^{-2}$. The D Ly- α emission rate would then be (1.0 ± 0.4) kR, not 2.5 kR (Fig. 2). 300 R of D Ly- α would imply a D/H ratio of $(5.0 \pm 2.1) \times 10^{-3}$ at 100 km in the Paxton *et al.* hydrogen model.

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BERTAUX AND CLARKE REPLY—All the arguments presented by Donahue are sensible, but each allows room for discussion. Rather than going into the details here, we suggest another test of the presence of D atoms in the upper atmosphere of Venus. These atoms should produce an important limb brightening, just over the altitude at which CO_2 becomes transparent (horizontally) to Ly- α radiation, at about 125–130 km of altitude. This is because the D atmosphere is optically thin, whereas H atmosphere is not. With a

D/H ratio of 1.6×10^{-2} and the H model proposed by Donahue, this D Ly- α brightening should amount to 1–3 kR, superimposed on the smoother H Ly- α brightening. Perhaps the data collected by the ultraviolet spectrometer on board Pioneer Venus at low altitude could be re-examined carefully for the detection of such a deuterium limb brightening.

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Heart disease risks

SIR—Barker writes that “the geographical distribution of ischaemic heart disease is closely related to that of poor child health and development, indicated by high infant and child mortality about 70 years ago”¹. According to this aetiological model, people born in a relatively poor area, who subsequently move to a more affluent part of Britain will experience a higher incidence of ischaemic heart disease (IHD) than those who have always lived in the more affluent area. Conversely, regardless of where they eventually settle, people born and raised in more affluent areas will suffer less adult heart disease. Migration into, and within, Britain, provides the opportunity to test the hypothesis.

The British Regional Heart Study reported² a strong geographical gradient in the risk of a major IHD event in 7,735 middle-aged men, living in 24 towns in England, Scotland and Wales, who were followed up between 1978 and 1986. Regardless of where they were born, men examined in Scotland experienced the highest IHD risk, whereas those examined in the south of England had the lowest. Men who were born in the relatively poor part of Britain, north of a line drawn between the Severn estuary and the Wash, but who later moved to the comparatively affluent south, experienced a risk of IHD little different from men who had always lived south of the line. On the other hand, men born in the south who

moved north experienced a higher risk than those who remained in the south. Those who moved north faced an IHD risk similar to that of men who had always lived there. Furthermore, immigrants to Britain also adopted the IHD risk of the part of the country to which they moved, regardless of their birthplace.

These findings are consistent with other epidemiological studies of migrants. Between 1962 and 1966, immigrants to Australia from Italy, England and Wales had lower death rates from cardiovascular disease than Australian-born women and men³, but death rates rose progressively with increasing length of residence. Also, between 1970 and 1972 immigrants into England and Wales had, in general, IHD mortality rates intermediate between those of their original and new country of residence⁴.

Thus, environmental and cultural factors acting in adult life are of crucial importance in the development of coronary heart disease, independent of birthplace. It seems unlikely that geographical differences in IHD risk can be substantially explained by the prenatal and childhood environment.

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Spin glass in a whirl

SIR—Sourlas's use of spin-glass models as error-correcting codes (*Nature* **339**, 693; 1989) is a complex way of saying something essentially very simple: that binary arithmetic, as used in most conventional computers, is multiply connected. This is in contradistinction to Gray, or reflected, binary code, which is simply connected. This means, for example, that in conventional computers five switching operations, (binary 01111 to 10000), are required in changing from the binary code for 15_{10} to the code for 16_{10} ; there are thus factorial five (120_{10}) possible switching sequences. This fundamental flaw in conventional computation has been drawn to the attention of the UK Institution of Electrical Engineers in relation to the preparation of software intended for use in safety-critical situations, and also to the attention of the Combined Higher Education Software Team.

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Defective viruses and AIDS

SIR—Robin Weiss¹ and others²⁻⁵ have recently discussed the possibility that replication-defective viruses play a role in AIDS. Defective viruses may complement the host range and virulence of non-defective viruses with variant alleles of common genes or with unique genes⁶. Accordingly, defective human immunodeficiency virus (HIV) with variant *gag* or *env* genes could complement the host range and virulence of conventional HIV to generate a pathogenic virus complex^{1,2}. This notion is inspired primarily by feline and murine defective helper-virus complexes, which in animals a few weeks after experimental infection at high multiplicity, cause viraemia and some of the diseases that are now included in acquired immunodeficiency syndrome³⁻⁴.

If these animal systems were good models for AIDS, they would predict that humans develop AIDS soon after infection, carry high titres of the defective pathogen and helper HIV in primary lesions, and transmit AIDS with a latency of weeks or months. Furthermore, asymptomatic carriers of HIV should be protected against AIDS, and could in fact transmit a protective live vaccine. Yet none of these predictions is confirmed⁷. I suggest that even if a defective pathogenic virus arose in a human or in an animal outside the laboratory, it would not survive under natural conditions of transmission, and hence would not be relevant for the AIDS epidemic for the following reasons.

First, as the defective virus is unable to replicate on its own, pathogenicity of the complex would depend on double infection, and would thus be unstable. It is for this reason that defective viruses survive only on passage at high multiplicity of infection. Classical examples are the defective von Magnus influenza virus⁸ and the defective Bryan 'high titre' strain of Rous sarcoma virus^{9,10}. Indeed, a few passages at low multiplicity — termed biological cloning — are the orthodox method to rid virus stocks of defective variants. Because natural virus transmission is almost always based on low multiplicity of infection — perhaps often initiated by only one infectious unit — none of these experimentally generated virus complexes could survive in nature. This would be particularly true for HIV, whose titres *in vivo* are too low to be measured directly⁷.

Second, even in the rare cases where both a defective and a non-defective HIV were transmitted naturally or via a doubly infected cell to the same cell of a susceptible host, or if the defective version arose *de novo*, virus spread within the host would discriminate against the defective

component of the virus complex. As the helper virus is autonomous, it can out-replicate the defective virus by single infection, shifting the balance in favour of the non-pathogenic helper virus. Moreover, helper virus-infected cells would further discriminate against the defective pathogen by interference¹¹. Because the defective virus is packaged (pseudotyped) by the envelope proteins of the non-defective HIV, it would be either entirely (if it lacks *env*) or partially (if it lacks *gag*) excluded from all helper virus-infected cells.

Finally, all infections by non-defective HIV alone would further discriminate against natural transmission of the virus complex by antiviral immunity. Indeed, it was induction of interference and immunity against defective pathogenic retroviruses that earned non-defective helper viruses the term resistance-inducing factors¹¹. Even on simultaneous infection, the range of the hypothetical defective AIDS pathogen would be restricted by the same

antiviral immunity that restricts its helper virus⁹. In other words, a positive 'AIDS test' of an asymptomatic person, which detects antibody to HIV, should be considered a certificate of vaccination rather than a prognosis for death.

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Sulphate aerosols and climate

SIR—I proposed¹ that anthropogenic SO₂ emissions serve as a surrogate for enhanced marine dimethyl-sulphide (DMS) emissions to test the hypothesis of Charlson *et al.*² that marine DMS emissions regulate global cloud albedo and mean temperature by enhancing the number density of aerosol sulphate particles that serve as cloud condensation nuclei (CCN). I concluded that no influence of anthropogenic SO₂ emissions (confined predominantly to the Northern Hemisphere (NH)) of magnitude consistent with the sensitivities postulated in ref. 2 was found in inter-hemisphere comparisons either of present cloud contribution to planetary albedo or of 100-year temperature trends. None of the authors of the letters^{3,7,15,20} commenting on my paper, has come to grips with the fact that a major perturbation in emissions (tripling of NH gaseous sulphur emissions) should, if the hypothesis² is correct, have resulted in major perturbations of cloud contribution to albedo and mean temperature, neither of which is evident. By focusing on the trees of my argument, they have failed to perceive the forest.

Ghan *et al.*³ suggest that in continental air there may be an overabundance of CCN compared with over the ocean, making this air insensitive to incremental CCN derived from anthropogenic SO₂. In fact, the high concentrations of CCN commonly present in continental and continentally-influenced air may be due largely to human activity. There is abundant evidence (for example, refs 4,5) that the presence of anthropogenic sulphate greatly increases cloud droplet number densities in the continental air into which

it is emitted and in continentally-influenced air. While a nonlinear relation between cloud droplet and aerosol particle number density is evident, droplet number density is drastically enhanced in air influenced by industrial emissions relative to background continental air. Moreover, as air containing high CCN concentrations is diluted by mixing with cleaner air, the efficiency of the nuclei in nucleating cloud droplets would be expected to increase^{5,6}. A decreased efficiency at CCN concentrations of 1,000 or more per cm³ should not therefore be viewed as invalidating my argument.

A major premise of my article was that aerosol sulphate persists sufficiently long in the atmosphere for industrial SO₂, whose emissions are highly localized, to exert an influence throughout much of the NH marine atmosphere. I am surprised that Charlson *et al.*⁷ question this. Elsewhere Lovelock⁸ has taken it for granted in support of the assertion that tropospheric air in the NH and Southern Hemisphere (SH) does not freely mix, "as any observer travelling on a ship through tropical regions will readily perceive from the difference in clarity of the skies between the clean Southern and relatively dirty Northern hemispheres".

Charlson *et al.* question the suitability of the data presented in Table 2 of my paper to support the claim of widespread NH influence of anthropogenic sulphate, citing concern over comparing total non-sea-salt sulphate (n.s.s. SO₄²⁻) with fine (below ~1 µm, sub-µm) n.s.s. SO₄²⁻. Although I share this concern, I note that only 3 of the 11 SH data sets cited were for the sub-µm fraction, and that these measurements were explicitly compared with NH measurements that obtained much greater concentrations using the

same technique.

They suggest that high SO_4^{2-} concentrations at several mid-Pacific islands and at Barbados coincident with high concentrations of large-particle mineral aerosol represent SO_4^{2-} present on those large particles, which would therefore provide few CCN. The presence of dust was noted in my table and refs 9,10 as providing unambiguous evidence for long-range transport of airborne particles from continents, not as implying that the SO_4^{2-} was a dust constituent.

In noting that several of the NH locations, at times, exhibit extremely low n.s.s. SO_4^{2-} concentrations, Charlson *et al.* lose sight of my objective, which was to show that anthropogenic SO_2 frequently exerts a substantial influence on aerosol SO_4^{2-} concentrations throughout much of the hemisphere. This point is amply demonstrated by the aerosol SO_4^{2-} concentrations presented in my Table 2 and also by n.s.s. SO_4^{2-} concentrations in precipitation samples at remote locations and by time trends in n.s.s. SO_4^{2-} in NH glacial ice cores.

Ghan *et al.* suggest that because a significant proportion of SO_2 is oxidized by aqueous-phase reactions, the yield of CCN will be less from SO_2 than from DMS. Most DMS oxidized in remote marine environments is oxidized initially to SO_2 (ref. 11). There is, of course, ample evidence (for example, ref. 12) for formation of accumulation-mode sulphate aerosol from SO_2 as a consequence of gas-phase oxidation followed by gas-to-particle conversion and aerosol coagulation and growth. Moreover, new particle formation would be greatly enhanced at high SO_2 concentrations characteristic of industrialized regions, relative to remote marine locations, as H_2SO_4 production rates would be higher and the particle-nucleation rate increases sharply with increasing H_2SO_4 production rate¹³. Methanesulphonic acid, the alternative oxidation product of DMS, requires a much greater production rate than H_2SO_4 to initiate new particle formation¹⁵ and is thus unlikely to form new particles in remote marine atmospheres. Thus, if anything, the yield of CCN from anthropogenic SO_2 should be greater than from marine DMS, not less.

The possibility that soot particles incorporated into cloud droplets would decrease cloud albedo more than the increase due to enhanced cloud droplet number density has been examined¹⁴, and the amount of soot necessary to overcome a 30 per cent increase in albedo due to enhanced droplet number density is unrealistically large in comparison with measured visible absorption of atmospheric aerosols.

Ghan *et al.* and Henderson-Sellers and McGuffie¹⁵ question my choice of the cloud component of planetary albedo as

the observable to examine for indication of interhemispheric differences that might be attributable to anthropogenic sulphate, noting that this quantity, the product of fractional cloud coverage F_c and cloud albedo α_c might be expected to be higher in the SH because of a higher cloud fraction¹⁶. I share this concern. I chose to compare the cloud component of albedo of the two hemispheres and not cloud albedo or total albedo as suggested because, first, greater land area in the NH would contribute to a greater NH total albedo (due to greater clear-sky albedo over land than ocean) for reasons that have nothing to do with clouds. Second, the cloud component of albedo is the quantity that is directly relevant to the hypothesis under examination.

Ghan *et al.* express concern over the grouping of continental and oceanic albedo data. However, examination of the cloud component of albedo as was done for my Fig. 1 but for marine data only^{16,17} again yields no systematic NH excess, nor is a systematic excess exhibited in the total marine albedo in the NH.

Henderson-Sellers and McGuffie raise the concern that North American (and hence perhaps NH) fractional cloud cover, F_c , may have substantially increased over the past century, perhaps because of industrial activity, confounding my analysis. I reiterate that my analysis of cloud contribution to albedo is based only on modern data. Second, any increase in F_c would yield a value of $\alpha_c F_c$ that is high in the NH because of high F_c and not because of high α_c . The fact that $\alpha_c F_c$ was found to be less in the NH than the SH (or at least not greater) suggests that increased F_c is not a concern for my argument. Values of $\alpha_c F_c$ evaluated with Henderson-Sellers' data for F_c (ref. 19), while somewhat modified from those in my Fig. 1, are still systematically greater in the SH than in the NH. This result is insensitive to a 10 per cent increase in NH F_c .

Charlson *et al.* and Slingo¹⁸ point out that cloud albedo depends not only on the number density of cloud droplets but also on liquid-water path. The possible dependence on solar zenith angle suggested by Charlson *et al.* may be excluded in comparisons of equal north and south latitudes. However, the possibility that the liquid-water path, when clouds are present, is greater in the SH than in the NH, compensating for a greater NH cloud-droplet concentration due to anthropogenic SO_4^{2-} cannot yet be resolved.

Gavin *et al.*²⁰ question the adequacy of the temporal and spatial coverage of temperature data. Hansen and Lebedeff²¹ estimated the uncertainties associated with the mean temperatures determined for each hemisphere and for latitudinal bands within each hemisphere by pairing Northern and Southern Hemisphere stations to eliminate bias and by simulat-

ing spatial variability with a global circulation model. Their results are consistent with equal warming in both hemispheres and inconsistent with substantial lesser warming in the NH.

Charlson *et al.* call attention to an apparent NH cooling trend especially at high latitudes²². However, examination of those data shows this warming to be a short-duration fluctuation in the context of anthropogenic SO_2 (and CO_2) emissions. Oort *et al.*²³ state, with reference to their data analysis for marine-air and sea-surface temperatures extending back to the 1870s, that "the curves are surprisingly similar at all latitudes between 40° N and 40° S".

Gavin *et al.* suggest that regional cooling in the NH as seen by Jones *et al.*²³ in 1947–86 trends may be due to increased cloud albedo attributable to anthropogenic SO_2 emissions, consistent with the hypothesis of ref. 2. This seems unlikely, as the hypothesized sensitivity of cloud albedo and mean temperature to sulphate concentrations would imply a cooling due to regionally high SO_4^{2-} concentrations that is much greater than these regional departures from hemispheric trends. Also, the analysis of Jones *et al.* suggests that the North Pacific cooling anomaly is greater in extent and magnitude than the North Atlantic anomaly, whereas n.s.s. SO_4^{2-} in the North Pacific is less than that in the North Atlantic¹. It therefore seems unlikely that regional cooling anomalies over the past 40 years can be due to anthropogenic SO_2 emissions. The available temperature record is thus inconsistent with the hypothesis of Charlson *et al.*².

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The great calculator

Jack Meadows

The Works of Charles Babbage. Edited by Martin Campbell-Kelly. *Pickering & Chatto*, 17 Pall Mall, London SW1Y 5NB: 1988. Eleven volumes £500, \$995.

IN THE early 1840s, Tennyson published a poem containing the lines:
Every moment dies a man.
Every moment one is born.
 Charles Babbage immediately objected. It should be:
Every moment dies a man.
Every moment 1¹/₁₆th is born.

This story nicely suggests the two characteristics of Babbage (1792–1871) that most people have heard of — his love of numbers and computation, and his eccentricity. Both might better be seen as reflections of his determination to get things right. His long battle against street musicians, often cited as an example of his eccentricity, was actually a fight against noise pollution based on a determination that due legal process should be applied. His success in this battle was warmly applauded by his friends (see Vol. 11 of these *Works*). What is less well recalled than his computations and eccentricity is Babbage's catholicity of interests. This publication of his collected writings provides an opportunity to appreciate how widely he ranged.

At the age of 35 Babbage was left a considerable fortune by his banker father. He was also left with his father's worry that he would always be a dilettante. When at the end of the 1820s he was elected Lucasian Professor of Mathematics at Cambridge, he regretted his father was no longer alive to see him so established. In fact, Babbage threw himself into his varied endeavours with such enthusiasm that he could usually treat with professionals on equal terms. Although his life's work was unfinished, it was not the work of a dilettante.

This dedication was evident from the start, during his days as an undergraduate at Cambridge. He was already widely read in mathematics prior to entry, and took

along a French text on calculus for discussion with his tutor. He was told not to bother with it: no questions of that sort appeared in the examinations. This lack of official interest in Continental mathematics led Babbage and his friends to form the Analytical Society, intended both for the presentation of papers and the

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REASONS

Engine with a difference: now celebrated as the forefather of the modern digital computer, Charles Babbage designed his Second Difference Engine between 1847 and 1849. No attempt was made to construct the engine during his lifetime, but this trial piece, based on Babbage's drawings, was built recently by the Science Museum.

translation of Continental mathematics texts. When the first volume of the society's transactions was being prepared for publication, Babbage suggested it should be entitled "The principles of pure d-ism in opposition to the dot-age of the university". The phrase encapsulates what the Analytical Society was intended to do.

During the eighteenth century, mathematics in Britain was dominated by the memory of Newton. Amongst other things, British mathematicians retained a

dot notation in calculus, where the Continental mathematicians used 'd'. Their conservatism meant that, by the early nineteenth century, they lagged well behind their peers on the Continent. This was the situation Babbage and his friends were trying to change. The consequent developments were followed with interest in Cambridge. When Babbage translated a German mathematics text, a contemporary versifier claimed it marked the point.

When civilized, savage, Oxmanians and Hottentots

Shall set up the d's instead of those rotten dots.

By the time Babbage became Lucasian

Professor, the battle had been won (see Vols 1 and 11). Its consequences included not only the launching of the Mathematics Tripos as the accepted nineteenth-century test of intellect, but, more fundamentally, the resurgence of British mathematics and physics.

Alongside his interest in mathematics, Babbage was always concerned with experiment. In mechanical computation, these two pursuits were linked. Once, as he was sitting at the Analytical Society, one of his friends said, "Well, Babbage, what are you dreaming about?". To which he replied, "I am thinking that all these tables [pointing to some logarithms] might be calculated by machinery." In the 1820s, he began putting the idea into practice.

Babbage wanted to build a machine that would not only calculate any type of tabulated function, but would do so by a uniform process, which could thus be automated. For the latter purpose, he saw the value of using difference methods, because the

mechanism would then only need to carry out additions. Addition requires two activities: the addition of one digit to another and the carry over of any surplus to the next column. Babbage pointed out that these actions could be carried out using an appropriately geared set of cogwheels. By 1822, he had a prototype 'difference engine' working satisfactorily (see Vol. 2).

By the nineteenth century, mathematical tables had come to occupy an important place in commerce. Their

Science Museum

applications ranged from navigation at sea to life insurance. Automatic computation offered the possibility of overcoming two problems — one was the need to remove the sheer drudgery of the calculations; the other was to eliminate the correspondingly frequent errors. Babbage's engine could outpace human calculators and, by adding an automatic printer, he greatly reduced the likelihood of mistakes creeping in. Although there had been previous attempts to build calculating machines, Babbage's was the first to be fully automated.

Babbage now sought, and obtained, government funds to build a bigger and better difference engine. A section of the resultant machine was assembled in 1832 and worked well. The final version never appeared: partly because of difficulties with the craftsman involved, but also partly because Babbage went off on another track — the investigation of an analytical engine (see Vol. 3).

The difference engine was limited in the range of tables it could produce. Babbage wanted to produce a universal machine that could carry out any kind of calculation. This was the analytical engine. It is the design work he did on the analytical engine that entitles Babbage to be



Charles Babbage, aged 41.

claimed as the father of the modern computer, for he envisaged an all-purpose automatic machine controlled by punched cards. The cards were used both for data entry and to control the operations of the machine. With this level of complexity, and relying solely on toothed wheels for the operations, it is hardly surprising that a complete machine never appeared. It is more important that the principles were workable.

To the conservatives of the day, Babbage's work was simply a shocking waste of government money. He was classified as a radical (what would later be termed a 'liberal'). This appears most obviously in his writings on the decline of science in Britain (Vol. 7 — and see also Commentary, p.499 of this issue), in which he was concerned with several matters, in particular the state of the Royal Society. Although the arguments here revolved partly around personalities, there was an underlying theme of great importance — the need to professionalize British science. Babbage was in Berlin in 1828 when a major meeting of German scientists was being organized. His published review of the meeting was one of the stimuli for the creation of the British Association for the Advancement of Science. His attacks on the Royal Society and support for the BAAS played a part in gradually changing

attitudes to science in Britain.

Society activities took a great deal of Babbage's time. He helped found the Royal Astronomical Society in 1820, and was instrumental in establishing the Statistical Society in 1834. The link with both societies was his interest in the provision of tables. He became an expert on insurance and actuarial questions (Vol. 6) — whence came his comment to Tennyson.

In many ways, Babbage was a Victorian liberal before the Victorian age began. He believed strongly in the importance of individual enterprise. At the same time, he was sure that industry, rather than agriculture, would come to dominate the British economy. His book *The Economy*

of Machinery and Manufactures (Vol. 8) was a widely read and influential account based on his personal knowledge of the manufacturing industry. Just how wide his knowledge and his circle of acquaintances were can be judged from his writings on applied science and technology (Vols 4 and 5). The topics range through lighthouses, submarine navigation, gun-laying and cypher writing, not forgetting his favourite tabulations, such as the "Table of the relative frequency of occurrence of

the causes of breaking of plate glass windows".

In his beliefs Babbage was hardly an orthodox Christian, but he dismissed contemporary arguments that miracles could not be accepted by rational people. He used his own experience with calculating machines as a basis for his contentions, set forth in *The Ninth Bridgewater Treatise* (Vol. 9) — perhaps the first example of God as computer. Towards the end of his life, Babbage put together some autobiographical fragments (Vol. 11). He so enjoyed adding story after story that his friends feared the book, like his calculating machines, would never be completed. But, eventually, he came to write the conclusion, in which he took a last look at himself. His particular characteristic, he felt, was that, "every moment of my waking hours has always been occupied by some train of enquiry". This welcome edition of Babbage's work amply demonstrates just how many trains of enquiry he managed to pursue during his lifetime. □

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• A one-volume introduction to Babbage's writings was published earlier this year by Cambridge University Press. *Science and Reform: Selected Works of Charles Babbage* is edited by Anthony Hyman, and costs £35, \$59.50.

Some unhappy endings

Michael J. Benton

Mass Extinctions: Processes and Evidence. Edited by Stephen K. Donovan. *Belhaven, London: 1989. Pp.266. £32. To be published in the United States in September by Columbia University Press, \$45.*

IT IS nearly ten years since the mass-extinction industry began in earnest. Up to 1979, some 40 or 50 papers were published annually about particular mass-extinction events, about postulated causes and about grand all-encompassing theories. Then the famous 1980 paper by Luis Alvarez *et al.* triggered a revolution.

Alvarez and his colleagues argued that enhanced levels of the rare platinum-group element iridium, which they had found in a single stratigraphical section spanning the Cretaceous-Tertiary (K-T) boundary, indicated that a massive asteroid had hit the Earth. The K-T boundary coincides with the disappearance of the dinosaurs 65 million years ago. To many, the idea seemed preposterous, the stuff of science fiction and sensational journalism. But over the next few years enhanced iridium levels were found at nearly every K-T boundary in the world, more than 60 of them, in sediments that had been deposited on land, in shallow water and in the deep oceans. Further physical evidence has since come to light: glassy spherules, shocked quartz, isotope shifts, fossil 'soot'. The current output of papers reporting original research on mass extinctions and associated topics is now over 500 a year, possibly as many as 1,000.

Palaeontologists have had mixed views about the whole business, some of them grumbling about the encroachment of geophysicists, geochemists, astronomers, atmospheric modellers and mathematicians on their territory. Most have welcomed the high level of interest in the fossil record by scientists of all shapes and sizes, however, and Donovan's new book reflects the pivotal role of palaeontology in providing the data base that underpins all research on mass extinctions.

Toni Hoffman surveys the changing views of palaeontologists about mass extinction, essentially pre-Alvarez and post-Alvarez. More than half of the article is devoted to very personal accounts of nineteenth-century views, the rest to the argument that the periodicity of mass extinctions postulated by Raup and Sepkoski is a statistical artefact.

There is good coverage of the palaeontological (Stephen Donovan) and geochemical (Carl Orth) definitions of mass extinctions. The remaining contributions

are concerned with the nine major mass-extinction events, which range in time from the Precambrian to the Pleistocene.

The editor performs the interesting task of surveying the conclusions of those nine chapters. Do the gradualists or the catastrophists win? The gradualists, according to the present authors — there are eight votes for sea-level and temperature change against one for extraterrestrial impact. A different crop of contributors might well have produced the opposite result, but there seems little doubt that the evidence for impacts is not so easy to find at times of mass extinction other than the K-T event. This may be rectified by the intense search for such evidence that is now going on, or it may well turn out that mass-extinction events are all unique and

the result of different causes.

This is one of many books on mass extinctions that are now available. Of the multi-author volumes, it provides the best and most complete coverage of the palaeontological and geological evidence. A fuller account of the periodicity debate would have been helpful, as would a few more pictures of fossils. But Donovan has selected authors who are currently working on the topics they review, and there are refreshing insights into a great deal of the research they have in progress. It is also pleasing not to see the usual mass-extinction groupies showing their faces yet again in print. □

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Ad infinitum

Ian Stewart

The Book of Prime Number Records.
By Paulo Ribenboim. Springer-Verlag:
1989. Pp. 252. \$49.80, £32.

PRIME numbers have long been a source of fascination, and not just for professional mathematicians. They possess a baffling mixture of regularity and unpredictability. They are, as Paulo Ribenboim points out in his introduction, "like cousins, members of the same family, resembling one another, but not quite alike".

The Book of Prime Number Records is very loosely modelled on a volume produced annually by the manufacturers of a well known beverage; and according to Ribenboim it is intended to be used, by the mathematically minded, in a similar fashion. "Frankly, were I to read... that a brawl in one of our pubs began with a heated dispute concerning which is the largest known pair of twin prime numbers, I would find this highly civilized." But despite the self-deprecation, this book is much more than just a list of the trivia of primality. It is a moderately structured survey of a great deal of serious and important number theory. It is written for those who do not balk at lengthy and intricate formulae, but the subject-matter makes much of the book accessible to the non-professional.

Five main questions are addressed. How many primes are there? How can you tell whether a given number is a prime? Can primes be defined by formulae? How are primes distributed? What special kinds of prime have been invented?

There are, of course, infinitely many primes. Innumerable books on popular mathematics haul their readers through Euclid's proof of this basic fact; Ribenboim provides nine and a half different proofs, ranging from Euclid's cliché

to a topological proof discovered by H. Fürstenberg. The gist of Euclid's proof is that the product of all primes less than or equal to some bound n , plus 1, must be divisible by a 'new' prime that is greater than n . The first of many records appears immediately after: when is such a product itself a prime? The largest known case is when $n = 13,649$, leading to a prime with 5,862 digits.

The discussion enters considerably deeper water with the question of primality testing. When is a given number prime? If it is not, how can we find its prime factors? These questions have received a new impetus from their association with unbreakable codes, but their origins go back thousands of years and many famous or obscure names have contributed to their study. The important point to understand is that one can decide whether a number is prime *without* finding any factors. The simplest such test is Wilson's theorem: p is prime if and only if $(p-1)!+1$ is divisible by p . The size of the required computation makes this impractical, but recent advances have produced related tests for primality which can be applied routinely to numbers with hundreds of digits. Many of these ideas are described here. *En route*, we encounter the concept of a Carmichael number, which is a number that satisfies a whole battery of necessary conditions for primality while obstinately remaining composite. The largest known Carmichael number has 1,057 digits.

Formulae for primes do exist, though most of them are 'cheats' based on variants of Wilson's theorem. One that is not derived from the seminal work of Yuri Matijasevic on Hilbert's tenth problem, showing that there exist polynomials whose values are precisely the primes. In fact, Matijasevic proved that almost any sequence of integers can be determined by a polynomial. Several explicit examples of such polynomials are given here.

One of the most technical parts of prime-

number theory concerns the distribution of the primes. How many primes are there with given properties and within given limits? The results here are asymptotic; that is, they are approximations that become arbitrarily accurate for large numbers. For example the number of primes below a limit n is asymptotic to $n/\log n$. Analytical methods are widespread in this area. There are also unsolved questions, such as the twin primes problem. Do infinitely many pairs exist — p , $p+2$ — that are both prime? Nobody knows, but the largest known twin prime is $107,570,463 \times 10^{2,250} \pm 1$. Heuristic formulae for the distribution of twin primes are known which fit the available data well, and they suggest that the answer is infinite, but nothing in this area is rigorously proven.

A substantial amount of space is devoted to Goldbach's problem (is every even number a sum of two primes?), to Waring's problem (is every number the sum of boundedly many k th powers?), and the hybrid Goldbach-Waring problem (is every number the sum of boundedly many k th powers of primes?). The scope for records here is virtually unlimited; for example, every sufficiently large integer is a sum of 21 fifth powers, and of 23 fifth powers of primes.

Finally, there are various special kinds of primes. There are the Fermat primes (2^m+1 where $m=2^n$) and the Mersenne primes (2^p-1). The largest known prime is the Mersenne prime $2^{216,091}-1$. There are the regular primes, associated with Fermat's last theorem, the Sophie Germain primes (both p and $2p+1$ prime), the Wieferich primes, and the repunits 111...1 (the largest prime repunit has 1,031 1s).

I know of no other mathematics book quite like this one. It rambles, but not outrageously. It hops from genuine trivia such as repunits to deep and serious questions like Riemann's hypothesis. It does not always make the difference clear to the reader. It revels in the intellectual games that can be played among the primes but never really addresses the question of why they are worth studying. For all that, it is an immensely stimulating book and a worthy attempt to present a substantial part of the mathematical endeavour in a fresh way. Anyone who is fascinated by the strange quirks of whole numbers will find much enjoyment here. □
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• Publication of *Werner Heisenberg: Collected Works* continues its stately way with the appearance of Part II, Series A (original scientific papers) by Springer-Verlag. The contents include previously unpublished work to do with the German uranium project during the Second World War. The single-volume Series B (scientific review papers, talks and books) was reviewed in *Nature* 313, 826 (1985).

Stellar stuff

Michael L. Cherry

Neutrino Astrophysics. By John N. Bahcall. Cambridge University Press: 1989. Pp. 567. Hbk £40, \$75; pbk £14.95, \$24.95.

THE textbooks tell us that the Sun is powered by the fusion, deep within the solar core, of hydrogen into helium. Our Sun is a modest and unexceptional example, we are told, of the large numbers of main sequence stars in the Galaxy whose structure and evolution are well understood. Astronomers routinely and successfully use the predictions of stellar structure and evolution theory to explain many of the observed features of stars. But for the one star close enough for us to study in detail, direct observations of the fusion reactions give lower results than are predicted by standard models.

Typically, it takes some 10^4 years for photons to diffuse out from the Sun's interior. But neutrinos, since they interact only weakly with matter, get out directly and thus (presumably) provide the desired probe of the nuclear fusion processes in which they are created. Because the neutrinos interact so rarely, however, they are difficult to detect. Experiments must be massive (in order to provide enough sensitivity) and exceedingly free of background. Raymond Davis and his colleagues built the first (and for nearly 20 years the only) detector capable of measuring neutrinos from the Sun. It consists of 615 tons of C_2Cl_4 buried nearly a mile underground in the Homestake Gold Mine in South Dakota. Roughly once every two days, a neutrino is absorbed by a nucleus of ^{37}Cl , which is then transformed into ^{37}Ar . About every two months, in an elegant and now routine radio-chemical procedure, the resulting handful of argon atoms is extracted and counted. The observed rate of ^{37}Ar production, averaged over two decades, is only 25–30 per cent of the predicted rate.

A second experiment, involving the Kamiokande II water Cerenkov detector, has recently confirmed the low rate observed by Davis with a totally independent and equally impressive technique. Although the measurements are difficult to carry out, there appears to be nothing wrong with the experimental results. Instead, it seems that there is something wrong either with our understanding of the Sun or with our knowledge of neutrino physics. If the fault lies in the solar models, then there may be important ramifications for stellar evolution and for astronomy in general. And if the problem lies with the neutrinos, then these studies of low-energy solar neutrinos may in fact be trying to tell us something fundamental about electroweak physics at very high

Air bubbles — female workers at the Douglas Aircraft Company's plant in Long Beach, California assembling plexiglass canopies for warplanes around 1943. The canopies show a reflection of the factory's overhead lights. The picture is taken from *Beyond the Limits: Flight Enters the Computer Age*, published by MIT Press.

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

(grand unification) mass scales.

Like the experiments, the calculations aren't easy. They involve a blend of astronomy, nuclear physics, atomic physics, particle physics and hydrodynamics, and must be carried out with high numerical precision. Indeed, when one realizes how sensitively the calculated neutrino flux depends on the temperature of the solar core (for the observed neutrinos from 8B decay in the Sun, the flux is proportional to T^{25} !), it appears quite remarkable that such difficult and involved calculations agree with the experimental result as well as they do.

John Bahcall has been a leading and influential contributor to solar-neutrino calculations and theory since the field was young. In fact, nobody seriously interested in the subject should need to be told that his new book is worth reading. Bahcall spends the first half of the text authoritatively reviewing the theory and the models. His treatment reflects both his biases and much of the current interest and emphasis in the field. But even where the discussion is brief, he covers the main points, presents the conclusions and gives extensive references to the original papers. He reviews the possibilities for non-standard models thoroughly (making no attempt to hide his own strongly held opinions), but again the references more than make up for any unevenness in the treatment. A crucial question concerns the various uncertainties in the calculations: is the factor of 3–4 difference

between the chlorine measurement and the prediction a real problem? Bahcall's answer is a resounding yes. He also covers the hot topics of new neutrino physics (neutrino oscillations, for example) and supernova neutrinos in some detail.

Some of the excitement about solar neutrinos comes from the anticipation of imminent new experimental results. Two gallium experiments are now being prepared to look for the neutrinos from the fundamental proton–proton fusion reaction itself. These are by far the most abundant of all solar neutrinos, but are too low in energy to be seen by the existing detectors. The gallium measurement is expected to be crucial. Also, the Sudbury heavy water detector, to be built in Canada, promises to measure the neutrino energy spectrum and the neutral current contribution to the flux, both important pieces of information required to disentangle the effects of solar models and neutrino physics. Bahcall describes the existing and most of the proposed experiments, and finally includes a delightful history of solar-neutrino research, written with Ray Davis. *Neutrino Astrophysics* might more properly have been entitled *Solar Neutrinos*; but whatever the title, the book provides a thorough and expert summary of a field of intense current interest. □

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Sex in flies: the splice of life

Bruce S. Baker

The discovery that the primary transcripts of many genes are processed to produce more than one kind of messenger RNA focuses attention on the control of RNA processing as a developmental regulatory mechanism. In the fruit-fly *Drosophila melanogaster*, differential splicing of a hierarchy of regulatory genes determines sex, and thus the molecular biology of sex determination in the fruit-fly may lead to insights into the mechanisms by which alternative splicing is regulated.

THE determination of sex during embryogenesis in the fruit-fly *D. melanogaster* is transmitted through a hierarchy of regulatory genes to the terminal differentiation genes whose products are responsible for the sexually dimorphic characteristics of the adult (see refs 1–5 for reviews). The different activities of the regulatory genes in males and females are largely due to sex-specific differences in RNA splicing that lead to the production of functionally different transcripts in the two sexes. Recent molecular analyses have shown that the activities of the individual genes in this regulatory hierarchy are not only themselves controlled at the level of RNA splicing, but specify in turn the splicing pattern of the transcripts of the genes that function downstream of them in the hierarchy, which can thus be thought of as a cascade of RNA splicing reactions. The analysis of this regulatory cascade is not only elucidating the molecular basis of sexual differentiation but also contributing to our understanding of the mechanism of alternative RNA splicing, which is widely exploited as a means of gene regulation in higher animals.

The regulatory hierarchy

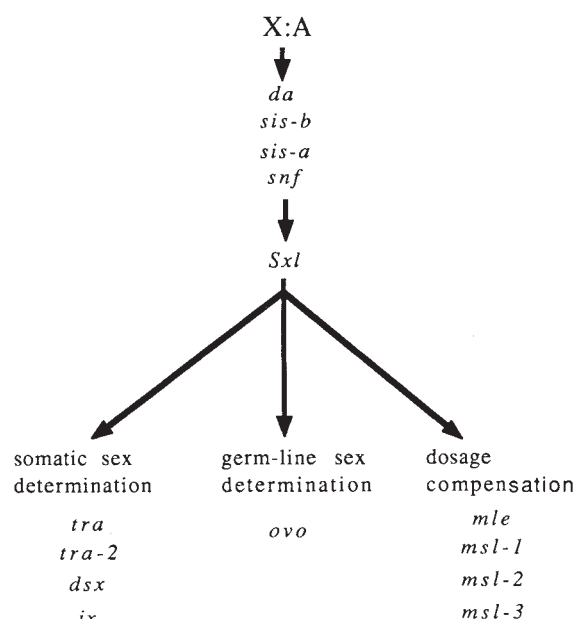
The primary determinant of sex in flies is the number of X chromosomes relative to sets of autosomes in a cell (the X:A ratio)^{6,7}. Flies with one X chromosome and two sets of autosomes are male, whereas individuals with equal numbers of X chromosomes and sets of autosomes are female; the Y chromosome has no role in sex determination. One of the first responses to the X:A ratio in females is the activation of the *Sexlethal* (*Sxl*) locus^{2,8}. The *Sxl* gene has two functions in female flies. First, it positively regulates its own expression and thus provides

each cell with a 'memory' of its sex⁹. Second, it controls the expression of other regulatory genes that govern somatic sexual differentiation, germline sexual differentiation and dosage compensation^{2,10–14}. In males, *Sxl* has no function and can be eliminated by mutation with no effect on phenotype¹⁵. Thus maleness would seem to be the 'default' state of the fly⁵. Whereas the *Sxl* locus controls all three aspects of terminal differentiation in females, each subsequent regulatory gene in the hierarchy controls only one (Fig. 1). I shall focus here on the genes regulating somatic sexual differentiation; for reviews of dosage compensation, see refs 16 and 17, and for recent work on germline sex determination, see refs 18–20.

It is not known how *Sxl* is activated by an X:A ratio of one; but genetic analysis has shown that both maternally and zygotically supplied gene products are required^{8,18–22}. One of the genes, *daughterless* (*da*), supplies a maternally encoded product that is necessary for *Sxl* activation and *da* has recently been shown^{23,24} to encode a protein with significant similarity to a mammalian enhancer-binding protein²⁵ and that may, therefore, be necessary for the initial transcriptional activation of *Sxl*. The *da* gene, like many others controlling crucial steps in early development, also has a vital zygotic function later, when it plays an essential part in the organization of the peripheral nervous system^{26,27}.

Molecular analysis of the products of the *Sxl* locus has shown that *Sxl* produces two temporally distinct sets of transcripts during embryogenesis^{10,28,29}. One of these is present only very early in embryogenesis and little is known about the transcripts, although it has been suggested that they may contribute to the initial activation of *Sxl*²⁹. The second set, which consists of three

FIG. 1 Overview of the regulatory cascade that depends on the number of X chromosomes relative to sets of autosomes (X:A ratio). The X:A ratio determines somatic sex, germline sex, and the X chromosome transcription rate (dosage compensation) by regulating the functioning of four groups of regulatory genes. The first group of regulatory genes (*da*, *snf*, *sis-a*, *sis-b*, and *Sxl*) functions to control all three processes. In females, in response to an X:A ratio of 1, the maternal product of the *da* gene and the zygotic functioning of the *snf*, *sis-a* and *sis-b* genes are necessary for the expression of *Sxl*. In males, *Sxl* is not expressed. Whether the *Sxl* gene is on or off determines the state of expression of the other three sets of regulatory genes that are specific to the processes of somatic sex determination, germline sex determination and dosage compensation. Dosage compensation in flies is achieved through the hypertranscription of the male's single X chromosome so that it produces as much RNA as the two X chromosomes in a female. The four male-specific lethal genes (*msl-1*, *msl-2*, *msl-3* and *mle*) are required in males for the hypertranscription of the X chromosome and have no known functions in females. Mutations in these four genes are lethal to males but have no effect on the phenotype of females. In females, the functioning of the *msl* genes is thought to be prevented by the action of *Sxl*; in males, where *Sxl* is inactive, the *msl* genes are active. Little is known about the process of germline sex determination, although one gene, *ovo*, has been identified that is required for the development of the female germ line. In loss-of-function *ovo* mutations, the female germ cells atrophy, whereas in males, germ cells develop normally. How the genes control somatic sex-determination function is explained in the text.



female-specific and three male-specific transcripts, appears somewhat later during embryogenesis, and is continuously present from then on. Developmental genetic studies have established that *Sxl* function is necessary during later stages for somatic sexual differentiation in females³⁰, so the second set of transcripts must encode at least some of the functional *Sxl* products in females. Analysis of the structure of the late *Sxl* transcripts explains why the loss of *Sxl* has no effect on males²⁹ (Fig. 3). All of the late male-specific *Sxl* transcripts contain an exon that is absent in the female-specific transcripts. The inclusion of the male-specific exon in the processed RNAs introduces a stop codon, truncating the open reading frame and resulting in non-functional transcripts (Fig. 3).

The *Sxl* locus regulates somatic sexual differentiation in females entirely through its control of the *transformer* (*tra*) locus³¹ (Figs 2 and 3). The *tra* gene also functions only in females³² and is regulated at the level of RNA splicing³³. The first exon of the *tra* primary transcript can be spliced to either of two acceptor sites in the complete primary transcript (Fig. 3). In females, the second site is used and its usage generates an mRNA with a long open reading frame encoding a functional *tra* gene product. The alternative site is used in male transcripts and in about half the female transcripts; its use once again

results in the introduction of stop codons giving rise to a mRNA with no detectable function.

The *tra* product collaborates with the *transformer-2* (*tra-2*) product to control the expression of the *doublesex* (*dsx*) gene in females³² (Figs 2 and 3), but because the morphological phenotypes of *tra*- and *tra-2* mutants are identical it has been difficult on the basis of genetics alone to establish how the two genes interact. The identification of a female-specific *tra* transcript has in effect provided a molecular phenotype which can be monitored, in *tra-2* mutant flies, for changes that would implicate *tra-2* in the control of *tra* (ref. 34). No such changes are found³⁴, however, establishing that *tra-2* acts either downstream of, or in parallel with, *tra*. The pattern of *tra-2* transcription makes the latter more likely: *tra-2* produces identical transcripts in the somatic cells of flies of both sexes, suggesting that it is expressed constitutively, but can function in somatic cells only in the presence of the *tra* product (refs 35 and 36, and W. Mattox and T. Goralski, personal communication).

The *dsx* locus, which is controlled by *tra* and *tra-2*, is active in both males and females³² (Fig. 2). In females, *dsx*, in conjunction with the gene *intersex* (*ix*), acts to repress the genes involved in the processes of terminal differentiation specific to males; the genes involved in female-specific terminal differentiation are not repressed, and so female sexual differentiation occurs. Conversely in males, *dsx* acts to repress the genes encoding female-specific terminal-differentiation functions; the genes encoding male-specific terminal-differentiation functions are not repressed and so male differentiation occurs. Thus *dsx* has active but opposite regulatory functions in the two sexes.

The molecular basis for the different functions of *dsx* in the two sexes is the sex-specific processing of its primary transcript^{37,38} (Figs 2 and 3). The primary *dsx* transcript is processed to give sex-specific mRNAs that have their first three exons in common but have different 3' exons and polyadenylation sites. In females, the three exons that are not sex-specific are spliced to an immediately adjacent, female-specific, fourth exon. In males, this exon is spliced out and the first three exons are joined instead to two male-specific exons located further downstream. The male-specific exons are eliminated from female transcripts by polyadenylation and cleavage at a site at the end of the fourth female-specific exon. The resulting sex-specific *dsx* mRNAs encode proteins with common amino-terminal sequences up to residue 397, but carboxyl termini of 30 female-specific amino acids in females and 152 male-specific amino acids in males. Neither protein encoded by *dsx* shows significant similarity to any protein in the current data bases.

Because the sex-specific *dsx* mRNAs differ in their polyadenylation sites, as well as in their use of 3' exons, the primary regulatory event generating these mRNAs could be the choice of either the polyadenylation site or the splice acceptor site. Although a definitive discrimination between these possibilities is not yet possible, indirect evidence favours the choice of splice acceptor site.

Trans control of splicing

Studies on the *tra* and *dsx* transcripts in flies mutant for other sex-determination genes have established that these genes control the sex-specific processing of the pre-mRNAs of genes below in the hierarchy³⁴. Thus the production of the female-specific *tra* transcript is dependent on wild-type *Sxl* function (but not on *tra* or *dsx* functions): in *Sxl* mutants, only the *tra* transcript common to both sexes is formed. Similarly, the presence of the female-specific *dsx* transcript is dependent on the functioning of the *Sxl*, *tra* and *tra-2* loci: flies mutant for these genes form only the male-specific *dsx* transcript. This is sufficient to explain all of the interactions seen between these genes in genetic studies. In the case of *tra*, this conclusion is reinforced by the demonstration that transcriptional control plays no part in *tra*'s sex-specific pattern of expression: a *tra* gene whose promoter has been replaced with the promoter from a heat-shock (HSP70) gene

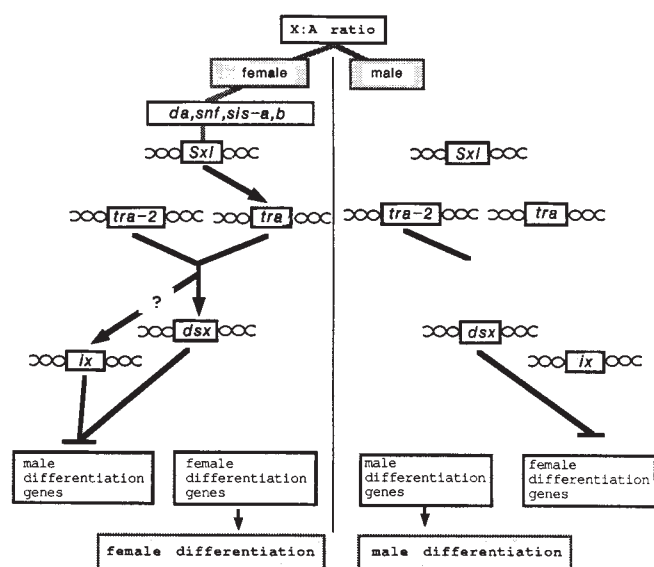


FIG. 2 The hierarchy of regulatory genes that controls somatic sexual differentiation in *Drosophila*, as inferred from genetic and molecular data. A line extending from a gene indicates that it is expressed. Arrows at the end of a line indicate a positive function. A bar at the end of a line indicates a negative function. The X:A ratio results in the female-specific activation of the *Sxl* gene. Activity of *Sxl* is required in females to control not only somatic sexual differentiation as depicted, but also germline sex determination and dosage compensation (see Fig. 1). Loss-of-function *Sxl* mutations in chromosomally female individuals are lethal (because of abnormalities in dosage compensation) and also lead to male development somatically. Mutations in the *tra-2* and *tra* genes also lead to chromosomally female individuals developing as males somatically. Mutations at these three genes lead to male development because their normal functions are necessary in females for the control of the *dsx* gene. The *dsx* gene has two functions. In females, the *dsx* product represses male development, whereas in males the alternative *dsx* product is expressed and functions to repress female development. In females that are mutant at the *tra*, *tra-2*, or *Sxl* genes, *dsx* is expressed in the manner characteristic of males, and male development occurs. Loss-of-function mutations at *dsx* result in the failure to repress either kind of sexual differentiation, with the result that intersexual individuals are produced as a consequence of the simultaneous occurrence of male and female somatic sexual differentiation. The product of the *ix* gene is required in females in conjunction with the *dsx* gene product for the repression of male differentiation. Mutations in *ix* affect only females and result in intersexual differentiation.

shows normal sex-specific regulation when reintroduced back into flies³³.

Similarly, the analysis of sex-specific transcripts provides a molecular basis for the suggestion⁵ that male differentiation is a default state of development. That is, in males there does not seem to be any active transmission of information between the different levels of regulatory genes in the sex-determination hierarchy: male-specific splicing patterns occur by default in the absence of female-specific gene products. Moreover, females mutant for *Sxl*, *tra* or *tra-2* genes process the *dsx* transcript into the male *dsx* mRNA, implying that females have the RNA processing machinery needed to carry out the male-specific pattern of processing of the *dsx* pre-mRNA, and that this corresponds to the housekeeping or default pattern of RNA splicing. Females differ from males because the products of *Sxl*, *tra* and *tra-2* genes modify the specificity of the housekeeping RNA splicing machinery so that the female-specific mRNAs of *Sxl*, *tra*, and *dsx* are produced.

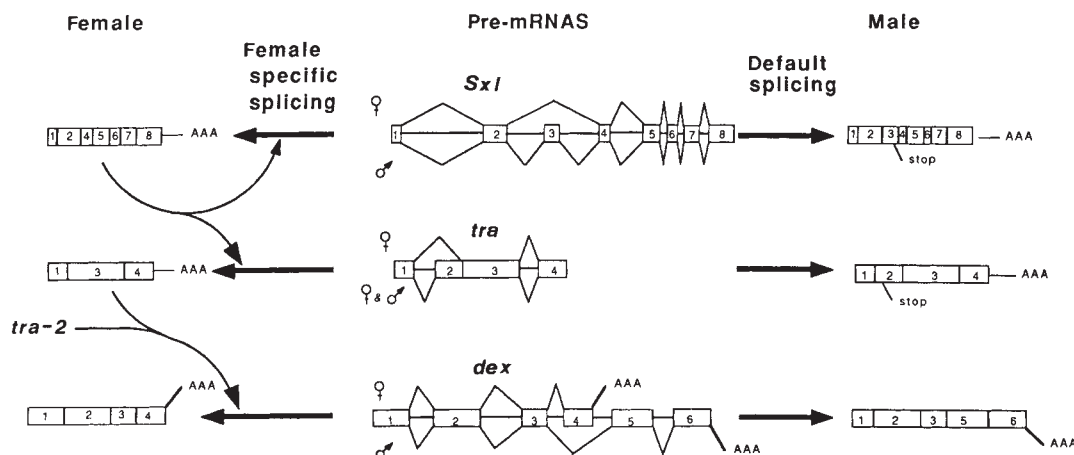
Insight into how these genes may act in females to regulate splicing of the pre-mRNAs of the genes downstream of them has come from the inferred amino-acid sequences of the proteins they encode. Each of the proteins encoded by the *Sxl* and *tra-2* genes has a domain of about 80 amino acids containing a motif of about 20 conserved residues which is characteristic of a family of single-strand-RNA-binding proteins (refs 29, 35 and 36; refs 39 and 40 for reviews). Included in this family of proteins are the main proteins of heterogeneous ribonucleoproteins (hnRNPs) and proteins that are found in small nuclear ribonucleoproteins (snRNPs), some of which are thought to be necessary for RNA splicing^{41,42}. These sequence similarities led to the suggestion that the female-specific *Sxl* and *tra-2* products interact respectively with the *tra* and *dsx* pre-mRNAs, to direct their processing into the female-specific mRNAs. In addition, the *tra* protein³³, the *tra-2* protein^{35,36}, and the U1 snRNP of relative molecular mass 70,000 (ref. 42), contain a region that

is rich in arginine and serine residues; such a region is also found in the protein encoded by the *suppressor-of-white-apricot* (*su(w^a)*) gene of *D. melanogaster*, which regulates its own splicing (see ref. 43, and ref. 44 for a review). These findings offer some insight into how *Sxl* could function to control the array of biological processes that it regulates (see Fig. 1).

As *Sxl* regulates somatic sexual differentiation by directing the female-specific splicing of the *tra* pre-mRNA^{33,34}, and because the amino-acid sequence of the *Sxl* product is similar to those of a family of RNA-binding proteins²⁹, then the other biological processes under the control of *Sxl* might also be controlled at the level of splicing. Presumably, therefore, the positive autoregulatory function of *Sxl* might entail the operation of the *Sxl* product on its own pre-mRNA to direct the female-specific splicing pattern. Similarly, *Sxl* may control dosage compensation by regulating the splicing of the pre-mRNA of one or more of the male-specific lethal genes, which must be active in males and inactive in females for proper dosage compensation^{45,46}. Thus, in females *Sxl* is proposed to direct a pattern of splicing of the pre-mRNA of one of these genes to produce a non-functional mRNA. In males, where *Sxl* function is absent, the default pattern of splicing occurs to produce a functional mRNA for this gene. In female germline *Sxl* presumably controls the splicing of pre-mRNA specifying a downstream regulatory protein necessary for female germline function.

It is not at first sight clear why there should be so many steps in the splicing cascade: why not eliminate the *tra* gene and use *Sxl* activity directly to control the splicing of the *dsx* pre-mRNA in females? One possible explanation is suggested by the finding that the *Sxl* product is relatively inefficient at directing the splicing of the *tra* pre-mRNA into the female-specific transcript: recall that in females about half of the *tra* pre-mRNA is spliced to produce the non-sex specific transcript^{31,47,48}. The *tra* and *tra-2* products, on the other hand, are efficient at directing splicing: the default (that is, male-specific) *dsx* transcript is not

FIG. 3 The patterns of splicing of the *Sxl*, *tra*, and *dsx* genes. The pre-mRNA (primary transcripts) of each of these genes are identical in males and females (central portion of figure) but are processed to produce male- and female-specific mRNAs. Boxes represent exons, horizontal lines introns. The male- and female-specific patterns of splicing and polyadenylation of the pre-mRNAs are depicted above and below, respectively, the structure of the primary transcripts. The mRNAs that are generated by sex-specific processing are shown to the left (female) and right (male). In males the primary transcripts of all three genes are spliced in the default pattern of splicing, whereas in females, alternative female-specific patterns of regulated splicing occur. The *Sxl* transcripts depicted represent the basic pattern of sex-specific splicing of the late *Sxl* transcripts. The male-specific transcripts all include exon 3, which introduces a stop codon into the open reading frame resulting in a non-functional mRNA. In the pattern of splicing that produces the female-specific mRNA, exon 3 is omitted and the mRNAs contain a long open reading frame. In addition to these sex-specific differences in splicing, there are alternative splicing differences found in both males and females which arise through the use of (1) alternative splice-site donors at the end of exon 1 and alternative acceptors at the beginning of exon 2 that generate diversity in the 5' untranslated leader, and (2) alternative splice acceptor sites in exon 5 that are in the translated portion of the female mRNA. The female-specific product of *Sxl* has two genetically defined functions in somatic sexual differentiation. First, it positively regulates its own activity; positive autoregulation is proposed to occur by an *Sxl*-encoded protein directing the female-specific pattern of splicing of *Sxl* pre-mRNA.



Second, the activity of *Sxl* in controlling the expression of other sex-determination regulatory genes occurs through its regulation of the activity of the *tra* gene. In females, *Sxl* directs the use of the female-specific splice acceptor site at the beginning of exon 2 of the *tra* pre-mRNA, leading to the production of an mRNA with an open reading frame able to encode the *tra* product. In males, and for about half of the time in females, the default pattern of splicing occurs, in which an alternative acceptor site is used to produce a non-sex-specific mRNA which has a stop codon in the open reading frame and is consequently non-functional. In the figure, for simplicity, the non-sex-specific *tra* mRNA has been omitted from the females' RNAs. The product of the *tra* gene functions together with the product of the *tra-2* gene in females to direct the female-specific pattern of splicing and polyadenylation of the pre-mRNA of the *dsx* gene. In females the first three exons of the *dsx* gene are spliced to a female-specific fourth exon to generate the female mRNA, whereas in males the default pattern of splicing occurs that joins the first three exons to the male-specific fifth and sixth exons to generate the male-specific mRNA.

detectable in wild-type females³⁸. Why the *Sxl*-directed splicing of the *tra* pre-mRNA is inefficient is not known (it could be because the *Sxl* protein has to regulate the splicing of transcripts from several genes). This level of efficiency, however, would not be acceptable in the splicing of *dsx* pre-mRNA, which must be very efficient if a sexually normal, fertile adult is to be formed. The male- and female-specific *dsx* products are mutually antagonistic: when both products are present, neither of them functions and flies develop as sterile intersexes similar to those produced by *dsx* null mutations^{45,46}. The mechanism of this interference is not understood (although it is known to occur at a level following the processing of *dsx* RNA (refs 6 and 38; R. Nagoshi and B.S.B., manuscript in preparation). Clearly, however, the demands on the *Sxl* and *tra* or *tra-2* products are different and may account for the existence of two steps between the X:A ratio and the regulatory genes that directly control the sex-specific phenotype.

Cis-acting sequences in regulated splicing

A priori there are two general ways in which the use of alternative splice sites could be regulated in primary transcripts of genes such as *Sxl*, *tra* and *dsx*. The splicing of a pre-mRNA begins with the formation of a complex of snRNPs, the spliceosome, on conserved sites at the ends of the intron that is to be removed. This complex is responsible for bringing together the donor site at the 3' end of the upstream exon with the acceptor site at the 5' end of the downstream exon. The choice of downstream exon thus depends on the acceptor site on which the complex forms. Therefore, the first way in which *trans*-acting factors could regulate splicing could be by preventing the use of the default acceptor site (the male-specific acceptor sites of the *Sxl* and *dsx* primary transcripts and the non-sex-specific acceptor site of the *tra* primary transcript), with the consequent use of the regulated acceptor instead. Alternatively, the *trans*-acting factors could act in the region of the regulated acceptor (in this case the female-specific acceptor) so as to make it the preferred acceptor site. Analysis of *cis*-acting mutants of *dsx*, *tra* and *Sxl* that perturb the patterns of splicing of their pre-mRNAs suggests that regulation of *Sxl* and *tra* may occur by blocking the default acceptor, whereas regulation of *dsx* may occur by enhancing the use of the regulated acceptor.

In the case of *Sxl*, there are five mutants in which the *Sxl* female product is inappropriately expressed (that is, the female acceptor is used) in males. All five mutations map to within one kilobase of the male-specific exon, suggesting that it is the disruption of the default splicing pattern in males which leads to the inappropriate use of the female acceptor site^{28,29}. This

implies, as the simplest possibility, that the wild-type *Sxl* product may function to prevent the use of the *Sxl* male-specific acceptor site, and that when this occurs the cellular housekeeping splicing machinery chooses the female acceptor instead. *In vivo* analysis of mutations generated *in vitro* in the region specifying the default acceptor of the *tra* primary transcript suggests that splicing of the *tra* transcript is regulated in the same way⁴⁹.

There are four *cis*-acting mutants that cause the constitutive expression of the *dsx* male function. In splicing the pre-mRNAs of these mutant genes the default (that is, male-specific) acceptor is invariably used (ref. 6; R. Nagoshi and B.S.B., manuscript in preparation). All four mutations map to within thirty to a few hundred nucleotides 3' of the regulated (female) acceptor. The simplest interpretation is that the products of *tra-2* and *tra* act on this region near the female-specific *dsx* acceptor to make it the preferred acceptor. In the absence of *tra-2* or *tra* function, or if this region is disrupted, the default (that is, male-specific) acceptor is used instead. The region where the *dsx* dominant mutants map contains six copies of a 13-nucleotide sequence which could be the recognition sequence for the *trans*-acting factors that regulate splicing of the *dsx* pre-mRNA³⁸. Further indication that the region of the female-specific *dsx* acceptor is important for regulated splicing is that this acceptor poorly matches the *Drosophila* polypyrimidine-tract acceptor consensus³⁸. Evidence that the pyrimidine deficiency of the female acceptor may be significant in *dsx* regulation is the finding that this feature of the acceptor is conserved in the *dsx* gene of *D. virilis*³⁸, which diverged from *D. melanogaster* some 60 million years ago.

There are no data so far that would indicate whether the products of the *Sxl*, *tra* and *tra-2* loci interact directly with the pre-mRNAs whose splicing they control, or act indirectly by, for example, associating with and modifying the specificity of an snRNP or another component of the spliceosome. At least in the case of the *dsx* primary transcript, where regulated splicing must be very efficient in females to prevent the production of sterile intersexes, the first mechanism seems more likely. That is, it seems easier to achieve this level of specificity by having the products of *tra* and *tra-2* associate with each *dsx* pre-mRNA molecule than to modify every copy of some component of the splicing machinery. Further experiments should conclusively determine whether or not the products of the *Sxl*, *tra* and *tra-2* loci interact directly with the pre-mRNAs of the genes downstream of them in the hierarchy. □

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Searches for low-temperature nuclear fusion of deuterium in palladium

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A series of experiments has been performed to determine whether nuclear fusion processes occur in palladium rods that have been electrochemically charged with deuterium. With a variety of metallurgical pretreatment procedures and different electrolytes, no evidence has been obtained for any excess enthalpy, neutron, gamma ray, tritium or helium production during electrolysis of D₂O with palladium cathodes.

In response to claims of unusual nuclear processes occurring at or near ambient temperature and pressure¹, we report the results of a detailed series of experiments on the behaviour of palladium rods that have been electrochemically charged with deuterium. Employing a series of metallurgical pretreatment procedures and several different electrolytes, we have not found evidence for any excess enthalpy, neutrons, γ -rays, helium or tritium. Experiments performed with sensitive neutron and γ -ray detectors have allowed us to place stringent upper bounds on the rate of nuclear fusion events occurring in these systems.

Preparation of electrolytic cells

We used several different sources of Pd in these experiments: 2.2-mm- and 3.8-mm-diameter cold-worked Pd rods and Pd sponge (>99.9% Pd) were obtained from David Fell, Inc. (Los Angeles); 1.0-mm-diameter cold-worked Pd rods (>99.9% Pd) were obtained from AT&T Bell Laboratories and from Aesar, Inc.; 0.25-mm-diameter wire (>99.99% Pd) was obtained from Engelhard Industries; a Pd ellipsoid, having a major axis of 3 cm and minor axes of 0.7 cm and ~0.44 cm respectively, was obtained by r.f. melting of a Pd ingot (>99.999% Pd) in an argon atmosphere. Portions of the 1.0-mm- and 2.2-mm-diameter rods were recast by melting the Pd at 1,600 °C for 5 min with either unregulated cooling to ambient temperature or by cooling at a rate of 50 °C min⁻¹ through the melting point. The recasting procedures were performed in Al₂O₃ tubes under 1 atm of CO(g) and yielded Pd rods of 2.1-mm- and 2.5-mm-diameter, respectively. In addition, the Pd sponge material was formed into 2.5-mm rods, after melting with an acetylene torch, by centrifugal casting. All cast rods were smoothed with sandpaper and ultrasonically cleaned in D₂O before use in the electrochemical cell.

We made a stock solution of 1.0M LiOD by adding a weighed amount of Li wire to 99.5% D₂O (Aldrich); immediately before use we diluted this stock solution to 0.1M LiOD with D₂O. Similarly, we prepared 0.1M LiOH from Li wire and deionized H₂O. In some cases, we pre-electrolysed the solutions before use, using Pt working and counter electrodes². The electrochemical cells for neutron, γ -ray and tritium measurements were typically 20-cm³ glass reaction vessels fitted with

polypropylene caps to prevent exchange of D₂O with ambient H₂O. The caps also had a small vent for the evolving gases, and the electrode leads were sealed with epoxy resin to the cap of the vessel. The extent of H/D exchange in the D₂O electrolyte was measured by ¹H NMR integration of the H₂O peak relative to a benzoic acid standard; in a typical electrolysis cell, the D₂O was found to contain 0.8 atom% ¹H after 540 h of operation.

The Pd rods were generally placed in the centre of the glass reaction vessel and were surrounded by a Pt-foil, wire or gauze counter electrode. A cathodic current was maintained from initiation until the end of all experiments to avoid outgassing of the D from the Pd rod. The potential of the Pd cathode during electrolysis was measured relative to a Pd-wire reference electrode charged with D to the α - β phase transition³; generally the Pd rods were charged to -1.5 V relative to this reference potential (see below) at current densities of 64 mA cm⁻², and the resistance-corrected charging potential was ~-0.8 V relative to the Pd/D reference. A charged specimen (0.025-cm-diameter $\times$ 2.3-cm-length rod, charged at 75 mA cm⁻² for >12 h) had an open-circuit potential of <-0.60 V relative to a Pd/D reference electrode, as determined by current-interrupt methods⁴ (5.0 ms after current interruption). Measurements of the volume of D₂ gas liberated from three separate electrochemically charged D/Pd samples (each 0.25-cm diameter $\times$ 2.5-cm length) were made by heating them with a propane torch, in a quartz tube preloaded with Ar, until gas evolution ceased. The D/Pd stoichiometries of 0.77, 0.79 and 0.80 obtained from these measurements were taken to be representative of the D/Pd stoichiometry for the charged cathodes used in this work. X-ray photoelectron spectroscopic (XPS) analysis of a 0.25 $\times$ 2.5-cm electrode, after two weeks under constant-current electrolysis conditions in 0.1M LiOD/D₂O, showed the presence of Pd and no signal above background in the Li region (that is, less than monolayer equivalents of Li in the 30-Å escape depth of the photoemitted electrons). We subjected samples to a variety of pretreatments, including recasting, vacuum annealing and anodization⁵. Also, current levels were varied to include oscillating-current pulses, abrupt current steps and/or constant currents for the duration of an experiment.

Neutron measurements

Table 1 lists the known exoergic nuclear reactions of deuterium (²H) with all stable or long-lived isotopes of hydrogen, helium and lithium, together with the energies released in the reactions. At low laboratory energies (a few keV), reactions (b) and (c) occur with about equal probability and with ~10⁷ times the probability of reaction (d)⁶. Reaction (e) occurs with ~10⁵ times the yield of (f). At the much lower energies relevant to the present work at ambient temperature, interaction-barrier considerations lead one to expect that reaction (a) should occur with the highest probability⁷, followed by reactions (b) and (c) (about equal probabilities), then (e), (f), (g), (h), (i), (j) (k) and

(1), in order of decreasing probability. Table 1 also lists the number of reactions per second that would be needed to produce an output power of 1 W for each of the reactions under consideration.

The neutron detector used in our measurements has been recently developed⁸, and is somewhat similar to that described in ref. 9. It consists of a 40-cm cube of polyethylene with a 10 × 10-cm open horizontal channel through the centre, in which the electrolysis cells were placed. Thermalized neutrons were detected with twelve ³He proportional counters embedded in the cube around the central channel. The cube was surrounded by a 0.6-mm-thick cadmium layer, a 10-cm-thick passive polyethylene shield, a 2π array of 2.5-cm-thick plastic scintillator paddles and a 25-cm-thick passive paraffin-wax shield. In some experiments, we used only 8 counters (14% efficiency), whereas in other experiments we used as many as 11 counters (20% efficiency); to account for this variation, we made appropriate calibrations and foreground/background comparisons for each run. Software cuts in the pulse height spectra were made to reject small signals from γ-rays and preamp noise, and higher energy α-particles. Further cuts were made on the timing spectra between the paddles and counters to reject cosmic-muon-induced neutron production inside the cube. The remaining background rate of 80–120 counts h⁻¹ was due to roughly equal contributions from alpha emission from the counter walls and from high-energy neutrons produced by cosmic rays outside the cube.

Table 2 summarizes a series of experiments designed to detect the emission of neutrons from the samples. In no case did we observe a statistically significant neutron count that exceeded the background level. Figure 1 displays the neutron detection rate as a function of time for a representative counting period. In this particular series of runs, background runs with dummy cells were interspersed between foreground runs for four fully charged cells. All runs depicted in Fig. 1, as well as all other runs performed, agreed with the average rate of ~610 neutrons per hour, within the combined statistical and systematic errors. We have thus established an upper limit on the net neutron rate (that is, foreground minus background) of 100 neutrons per

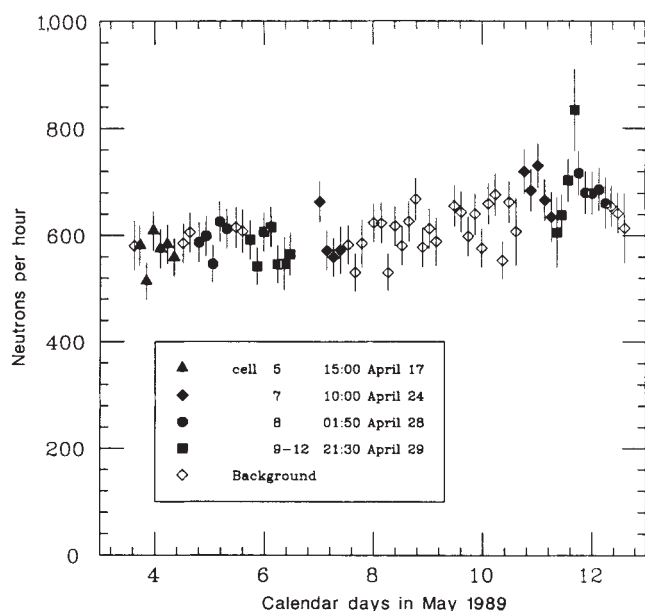


FIG. 1 Neutron rate for various runs with and without electrolysis cells. For each run, the data shown are the detector counts, divided by the efficiency, and the run duration (~3 h). The errors include only the statistical uncertainty; an estimate of the systematic uncertainty was calculated by determining the excess variance among background runs. The times in the inset refer to the initial application of cathodic current to each cell, and the current was maintained continuously through the analysis period.

TABLE 1 Low-energy deuteron fusion reactions

	Reaction	Energy release (MeV)	Reactions per second per 1 W output
a	$^1\text{H} + ^2\text{H} \rightarrow ^3\text{He} + \gamma$	5.5	1.1×10^{12}
b	$^2\text{H} + ^2\text{H} \rightarrow ^3\text{He} + \text{n}$	3.3	1.9×10^{12}
c	$^2\text{H} + ^2\text{H} \rightarrow ^3\text{H} + ^1\text{H}$	4.0	1.6×10^{12}
d	$^2\text{H} + ^2\text{H} \rightarrow ^4\text{He} + \gamma$	23.8	2.6×10^{11}
e	$^2\text{H} + ^3\text{H} \rightarrow ^4\text{He} + \text{n}$	17.6	3.6×10^{11}
f	$^2\text{H} + ^3\text{H} \rightarrow ^5\text{He} + \gamma$	16.7	3.7×10^{11}
g	$^2\text{H} + ^3\text{He} \rightarrow ^4\text{He} + ^1\text{H}$	18.4	3.4×10^{11}
h	$^2\text{H} + ^4\text{He} \rightarrow ^6\text{Li} + \gamma$	1.5	4.2×10^{12}
i	$^2\text{H} + ^6\text{Li} \rightarrow ^2\text{He} + ^4\text{He}$	22.4	2.8×10^{11}
j	$^2\text{H} + ^6\text{Li} \rightarrow ^7\text{Li} + ^1\text{H}$	5.0	1.3×10^{12}
k	$^2\text{H} + ^6\text{Li} \rightarrow ^7\text{Be} + \text{n}$	3.4	1.8×10^{12}
l	$^2\text{H} + ^7\text{Li} \rightarrow ^2\text{He} + \text{n}$	15.1	4.1×10^{11}

hour at the 95% confidence level. For the $0.22 \times 10\text{-cm}$ Pd rod, this corresponds to an upper limit of 0.070 neutrons per cm³ Pd per s, which contrasts with the specific neutron flux of 3.2×10^4 neutrons per cm³ Pd per s reported in previous work¹. For a D/Pd ratio of 0.80, the 1σ upper bound of 73 neutrons per cm³ Pd per h for the $0.44 \times 3\text{-cm}$ Pd ellipsoid (Table 2) corresponds to an upper limit, at the 95%-confidence level, of 1.5×10^{-24} D(d, n) fusions per d-d pair per second.

Gamma ray measurements

We detected γ-rays using a high-purity intrinsic-germanium detector (150 cm³) and either a 7.6-cm-diameter × 7.6-cm-length NaI scintillator or a 15 × 15 × 25-cm NaI scintillator. The detectors and source cells were shielded by 10 cm of lead. In the later runs, a plastic scintillator paddle was used to reject muon-coincident events.

Table 2 presents the results of various runs; no statistically significant evidence was obtained for γ-ray production from any of the electrolysis cells. The entire γ-energy foreground spectrum from 30 keV to 6 MeV is displayed in Fig. 2; for all of the Pd electrolysis cells, both the foreground and background spectra were virtually identical to this spectrum. Our sensitivity places a conservative upper limit of <200 fusions per hour from reaction (a) because of the absence of the expected peaks from a 5.5-MeV γ-ray. Also, no γ-rays at 23.8 MeV have been seen in long-duration NaI-detector runs (Table 2). Furthermore, we have observed no excess bremsstrahlung or annihilation radiation which would be expected from reaction (d) if 23.8-MeV e⁺e⁻ pairs were emitted¹⁰ instead of 23.8-MeV γ-rays. The Ge-detector gamma-ray spectra also showed no evidence for γ-ray lines from the Coulomb excitation of the various Pd isotopes by protons and/or α-particles from reactions (c), (e), (g), (i), (j) or (l).

We also note that an authentic 2.223-MeV γ-ray signal originating from thermal-neutron capture in hydrogen (2 kHz ²⁵²Cf source placed in a H₂O bath) and observed with a 7.6 × 7.6-cm NaI detector has a much larger width (as a result of the intrinsic instrumental resolution limitation of NaI) than the γ-ray spectrum presented in previous work¹, and also displays a Compton edge just below the full-energy (2.223 MeV) peak that is missing in the published spectrum. For these reasons, we can conclude that the published peak¹ does not arise from thermalized neutrons. During the course of our studies, an independent analysis of the γ-ray peak was performed by Petrasso *et al.*¹¹, who reached similar conclusions.

If the known ²H + ²H reactions (b), (c) and (d) were to generate 10 W of power¹, with their known relative yields, ~8 × 10¹² neutrons s⁻¹, ~8 × 10¹² tritium atoms s⁻¹ and ~8 × 10⁵ 23.8-MeV-γ-rays s⁻¹ should be produced, respectively (see Table 1). Similarly, 10 W of power generated by reaction (a) should produce ~10¹³ 5.5-MeV-γ-rays s⁻¹. Alternatively, 10 W of power

TABLE 2 Summary of neutron and photon detection experiments

Cell no.	Cathode dimensions* (cm)	Comments	Electrolyte	$t_a^\dagger$ (h)	γ Detection‡			n Detection	
					Start§ (h)	upper limit		Start § (h)	upper limit (n per cm ³ Pd per h)
						5.5 MeV (γ per cm ³ Pd per h)	23.8 MeV (γ per cm ³ Pd per h)		
1	0.0254 × 0.2	99.99% Pd¶ no high I or redox activation	1.0M LiOH, D ₂ O	0.45	0**	**	**	—	—
2	0.0254 × 0.2	99.99% Pd¶ no act.	1.0M LiOH 50:50, D ₂ O:H ₂ O	0.45	0**	2.2 × 10 ⁶	—	—	—
3	0.0254 × 8	99.99% Pd¶ 300 °C, 10 ⁻⁶ torr 2 h	0.1M LiOD, D ₂ O	0.45	—	—	—	0	2.9 × 10 ⁴
4	hollow cylinder o.d.=0.52, i.d.=0.47 l=2.5	>99.9% Pd†† no act.	0.1M LiOD, D ₂ O	10	0	620	—	51	820
5	0.1 × 3.7	99.99% Pd‡‡ 350 °C, Ar 3 h	0.1M LiOD, D ₂ O	6.9	14 204	4,700 3,000	4.2 × 10 ⁵ 4.0 × 10 ⁵	0 38 93 465 559	3,200 2,800 3,600 2,900 4,700
6	0.22 × 10	pulsed electrolysis§§ >99.9% Pd 300 °C, 10 ⁻⁶ torr 2 h	0.1M LiOD, D ₂ O	33.6	570	1,100	—	559	4,700
					0	350	2.9 × 10 ⁴	34	210
					86	240	3.0 × 10 ⁴	134	200
					258	470	3.0 × 10 ⁴	278	180
					421	140	3.4 × 10 ⁴		
7	0.25 × 0.8	>99.99% Pd cast*** no act.	0.1M LiOD, D ₂ O	43.4	627¶¶	220	1.3 × 10 ⁴		
					33	1,800	2.9 × 10 ⁵	223	1,700
					193	1,500	2.9 × 10 ⁵		
8	~0.44 × 0.7 × 2.95	>99.999% Pd remelted*** 300 °C, 10 ⁻⁶ torr 3 h	0.1M LiOD, D ₂ O	~130	291¶¶	3,200	7.4 × 10 ⁴		
					36	70	1.2 × 10 ⁴	161	73
					180¶¶	85	3.3 × 10 ³	328	110
9-12	0.25 × 2.5	>99.9% Pd cast††† 300 °C, 10 ⁻⁶ torr 12 h	0.1M LiOD, D ₂ O	43.4	131¶¶	420	3.5 × 10 ³	140	130
		stepped current‡‡‡			227¶¶	160	3.5 × 10 ³	158	200

* Cathodes were rods except where otherwise noted. Dimensions indicated are: diameter × length.

† The activation time, $t_a = r^2/D$, where r is the radius of the cathode and $D = 10^{-7} \text{ cm}^2 \text{ s}^{-1}$.

‡ Photon counting experiments were performed using a Ge detector and a 7.6 × 7.6-cm NaI scintillation detector in parallel, except where otherwise noted.

§ Time in hours at which either photon or neutron counting was initiated, measured from the initiation time of the cell. Typical durations for photon and neutron counting sessions were ~6 h.

|| The estimated upper limit for neutron or photon flux from each cell expressed as the flux per unit Pd-cathode volume: For $n_t > n_b$, flux = $[(n_t - n_b) + (\sigma_t^2 + \sigma_b^2 + \sigma_{\text{sys}}^2)^{0.5}] / (\epsilon V_c)$; for $n_t < n_b$, flux = $(\sigma_t^2 + \sigma_b^2 + \sigma_{\text{sys}}^2)^{0.5} / (\epsilon V_c)$, where n_t and n_b are the foreground and background count totals, σ_t and σ_b are the respective standard deviations, σ_{sys} is the estimated systematic error, ϵ is the detector efficiency and V_c is the volume of the Pd cathode. $\sigma_{\text{sys}}/n_t \approx 10\%$ (neutrons); $<6\%$ (photons).

¶ Englehard Inc.

** Only the Ge detector was used for these experiments.

** Exact estimate of upper limit for these runs is unavailable, but approximates that for subsequent photon counting runs.

†† Manufacturer unknown; the composition of the cathode was assayed using energy dispersive X-ray analysis.

‡‡ Johnson Matthey Inc.

§§ The current density of the cathode was pulsed between 70 mA cm⁻² and 350 mA cm⁻² at 1 Hz.

||| David Fell Co.

¶¶ A 15 × 15 × 25-cm NaI scintillation detector was employed in parallel with a Ge detector.

*** Pd rod cast from a melt of >99.999% Pd which was solidified at a cooling rate of 50 °C min⁻¹ through T_m .

*** Irregularly shaped Pd ingot was formed by remelting of 99.999% Pd and solidified at a cooling rate of >200 °C min⁻¹ through T_m .

††† Centrifugal casting was employed to prepare rods from Pd obtained from '|||' above.

Cells 9-12 were photon and neutron counted simultaneously.

‡‡‡ The current density was stepped from 64 mA cm⁻² to 320 mA cm⁻² immediately before the foreground neutron count.

from reaction (e) would produce $\sim 4 \times 10^{12}$ neutrons s⁻¹. If the energy for reaction (d) could be converted to phonons by some unknown mechanism, as has been suggested¹², the coupling between the excited ⁴He compound nucleus and the lattice would have to be electromagnetic. The same electromagnetic field of the excited ⁴He nucleus must also cause the emission of γ -rays and e⁺-e⁻ pairs, which would have been detected in our experiments. Although reaction (i) would not necessarily

yield external radiation, the small difference in reduced masses between ²H + ⁶Li and ²H + ⁷Li makes it extremely unlikely that reaction (i) would be totally suppressed and neutrons would be detected as the product of ²H + ⁷Li fusion events. Our measurements therefore force us to conclude that none of these known nuclear fusion reactions is occurring at a substantial rate (conservatively, <100 per s per cm³ Pd) under our experimental conditions.

Tritium measurements

We have also analysed the electrolyte for production of excess ^3H that might result from reaction (c) in Table 1. Tritium measurements were performed on a Beckman Instruments LS-5000TD scintillation counter using a Beckman Ready-Gel scintillation 'cocktail'. The background tritium level of our D_2O feedstocks was 148 ± 12.2 decays $\text{min}^{-1} \text{ml}^{-1}$ (d.p.m. ml^{-1}). Electrolysis at 64 mA cm^{-2} of a $0.22\text{-cm-diameter} \times 10\text{-cm-length}$ Pd rod for 458 h yielded a final ^3H activity of 162.3 ± 12.7 d.p.m. ml^{-1} , whereas a separate run with a $0.25\text{-cm-diameter} \times 0.8\text{-cm-length}$ rod for 345 h at 64 mA cm^{-2} yielded a final ^3H activity of 149.5 ± 12.2 d.p.m. ml^{-1} . Some increase in the ^3H level of the electrolyte would be expected due to the isotope separation factor of $^3\text{H}/^2\text{H}$ at Pd, but this ratio is evidently sufficiently close to unity in $0.1\text{M LiOD}/\text{D}_2\text{O}$ that little detectable ^3H buildup occurs over the time periods of our experiments. The specific tritium production rate of $(5 \pm 7) \times 10^3$ ^3H per cm^3 Pd per s observed for the $0.22 \times 10\text{-cm}$ rod is far lower than the specific rate of $1\text{--}2 \times 10^5$ ^3H per cm^3 Pd per s reported in previous work¹. Preliminary results reported by other groups have detected larger levels of ^3H from Pd cathodes in D_2O (ref. 12), but we note that scintillation-cocktail chemiluminescence of unneutralized basic solutions might be responsible for these results.

He measurements

Results claiming high ^4He content (ref. 13; B. S. Pons on 'Science Journal', Public Broadcasting Network, United States and various newspaper publications) in the evolved gases at Pd cathodes have prompted us to investigate the composition of the gases in the headspace of our electrolysis cells. The analysis was performed with a VG model 7070 E magnetic-sector mass spectrometer. The instrument was operated in the electron-ionization mode (70 eV) at a mass resolution of 1,000. The gas mixtures that we used as calibration standards were D_2/O_2 (2:1), D_2/O_2 (2:1) containing 1,100 p.p.m. ^4He and D_2/O_2 (2:1) containing 8 p.p.m. ^4He . Samples of 3.0 ml (at a nominal pressure of 1 atm) from these calibration mixtures, from a sample of the gas in the headspace of a Pd/ D_2O cell ($0.2\text{-cm-diameter} \times 10\text{-cm-length}$ Pd rod) that had been operating at 64 mA cm^{-2} for >300 h, and from a sample of the ambient air in the laboratory, were injected using a gas-tight syringe for analysis. We averaged together sixty 2.5-s scans from mass 0–60 for each run.

When care was taken to exclude laboratory air from the gas

samples of the Pd/ D_2O headspace, no He was observed above the instrumental background (<1 p.p.m. relative to total gas content of 2:1 D_2/O_2). We used the Ar^+ signal at $m/e = 40$ a.m.u. as an indication of the amount of air in these samples; the relative intensity of the Ar^+ to $^4\text{He}^+$ peak ranged from 2,000–4,000 in the ambient air. A quantitative analysis indicated that the ambient air in our laboratory contained 4 ± 1 p.p.m. ^4He . We also note that the lifetime of He inside Pd/ $\text{D}_{0.65}$ samples is known to be >12 yr (R. C. Bowman Jr, personal communication and ref. 14); thus, our inability to detect He in the headspace is in accord with previous work. We analysed the He content of a $0.25\text{-cm-diameter} \times 2.5\text{-cm-length}$ Pd rod (sample no. 11 in Table 2, which had been used in $0.1\text{M LiOD}/\text{D}_2\text{O}$ for 158 h at 64 mA cm^{-2} , followed by 4 h at 320 mA cm^{-2} , followed by 169 h at 64 mA cm^{-2}) by melting the Pd and performing a mass spectroscopic analysis on the evolved gas. This measurement indicated no detectable ^3He or ^4He , above the instrumental background ($<8 \times 10^{11}$ He per cm^3 Pd). We also note that the previously claimed levels of ^4He (1–10 p.p.m. relative to D_2 for a $0.10 \times 10\text{-cm}$ rod at 64 mA cm^{-2} current density (C. Walling, personal communication and ref. 12)) are 30–300 times the expected ^4He yield from the reaction $^2\text{H} + ^2\text{H} \rightarrow ^4\text{He} + 23.8 \text{ MeV}$ for the reported excess power of 0.079 W (ref. 1).

Excess enthalpy measurements

We have also performed calorimetric experiments to determine the heat produced during electrolysis of D_2O and H_2O at Pd. The measurements were performed using the isoperibolic calorimetry mode (a constant-temperature system with a well defined heat loss rate to a constant-temperature bath) of a Tronac, Inc. (Orem, Utah) Model 1250 calorimeter equipped with a 60 l bath. Some experiments were also performed in a 5 l constant-temperature unit using a 30-cm^3 interior-volume dewar flask, with the dewar walls containing 1 atm of air. The dewar held, typically, 30–50 ml of electrolyte and was fitted with a small vent for evolved gases and had sealed inlets for the cell leads. The temperatures of the baths were maintained by active heating/cooling feedback at $27.00 \pm 0.01^\circ\text{C}$ (60 l bath) and $25.00 \pm 0.01^\circ\text{C}$ (5 l bath), and the temperatures of the electrolysis cells were monitored relative to the thermocouples immersed in the constant-temperature baths. The electrolyte was vigorously stirred with a motor; the stirring rate was maintained as constant as possible and the Joule heating as a result of stirring the

FIG. 2 Gamma-ray spectrum obtained from cell 5 in Table 2 using a Ge detector. Each channel has a width of 1.84 keV. The current was pulsed at 1 Hz from 70–350 mA cm^{-2} ; the foreground analysis time was 41 h. All γ -ray lines correspond to well-known transitions in the ^{238}U and ^{232}Th decay chains and ^{40}K ; no differences between foreground and background spectra were found for this run or for any other γ -ray run in Table 2. Major lines in the spectrum are labelled; also noted are the locations of recoil-corrected full-energy, first-escape and second-escape peaks from the γ -ray in reaction (a), Table 1. An energy window which includes these peaks has an efficiency of 2.9% in our geometry.

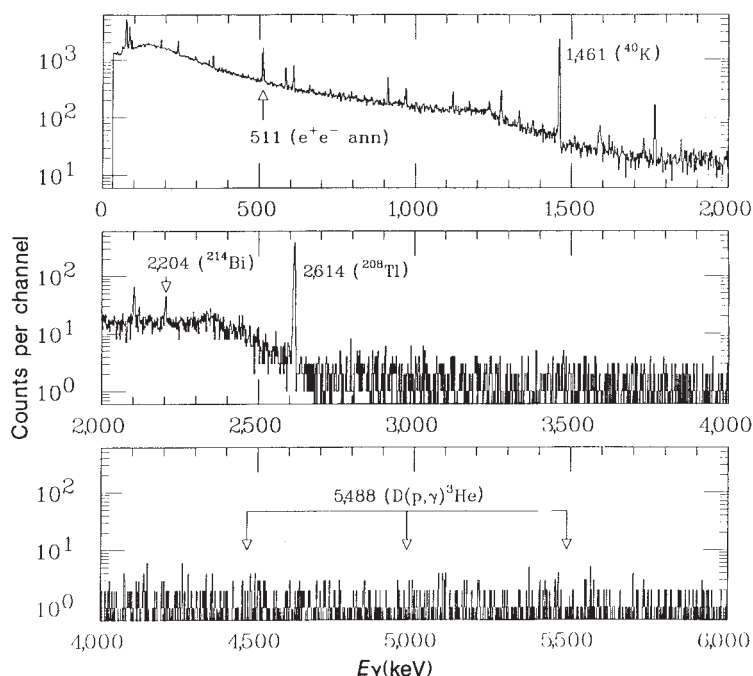


TABLE 3 Calorimetric data

(a) Cast Pd rod (0.21 × 2.1 cm), 0.1M LiOD/D₂O

	Time (h)	Current density (mA cm ⁻²)	Electrolysis power (W)*	Resistor power (W)	Total power (W)†	Temperature, <i>T</i> _{cell} (°C)‡	Heating coefficient (°C/W)§
A	14.7	108	0.463	0	0.463	31.80	14.0
	16.0	74	0.255	0.212	0.467	31.82	14.0
B	63.7	74	0.231	0.211	0.442	32.04	15.2
	66.0	110	0.429	0	0.429	32.01	15.6
C	88.7	110	0.407	0.212	0.619	34.69	15.2
	94.5	140	0.607	0	0.607	34.64	15.4
D	113.2	72	0.222	0.211	0.433	32.13	15.8
	115.0	108	0.426	0	0.426	32.08	15.9
E	161.0	140	0.595	0	0.595	34.69	15.8
	164.5	115	0.436	0.164	0.600	34.71	15.7

(b) Drawn Pd rod (0.38 × 2.4 cm), 0.1M LiOH/H₂O

Time (h)	Current density (mA cm ⁻²)	Total power (W)	Electrolysis power (W)¶	Heating coefficient (°C/W)*	Δ <i>T</i> _{th} (°C)**	Δ <i>T</i> _{th} (°C)††	Δ <i>T</i> _{meas} (°C)‡‡
3.6	8	0.064	0.029	12.6 ± 0.3§§	0.81 ± 0.02	0.37 ± 0.02	0.35 ± 0.05
20.7	60	0.668	0.405	11.7	7.8	4.7	4.67 ± 0.05
47.5	60	0.646	0.384	13.1 ± 0.3§§	8.5 ± 0.3	5.0 ± 0.2	5.45 ± 0.05

* Electrolysis power = (E - 1.54) × I.

† Total power = electrolysis power + resistor power.

‡ Bath temperature = 25.00 ± 0.02 °C; temperature rise in cell as a result of stirring is 0.30 ± 0.05 °C.

§ Heating coefficient = [*T*_{cell} - (25.00 + 0.30)]/Total power.

|| Total power = E × I.

¶ Electrolysis power = (E - 1.48) × I.

* Heating coefficients determined at the given electrolysis power, obtained from calibrations with a resistor using: heating coefficient = Δ*T*/(resistor power).** Δ*T*_{th} = heating coefficient × total power.†† Δ*T*_{th} = heating coefficient × electrolysis power.‡‡ Δ*T*_{meas} = *T*_{cell} - (*T*_{bath} + *T*_{stirrer}); *T*_{bath} + *T*_{stirrer} = 25.30 ± 0.05 °C.

§§ From three determinations.

solution was measured (~0.3 °C) and accounted for in all measurements. In the calorimetry measurements, a fixed power was applied to a resistive load that was included in the cell, while a known power (at constant current density, 64 mA cm⁻²) was simultaneously applied to the Pd/Pt circuit. This condition was maintained until the temperature of the cell reached a constant value *T*_{cell}; then the power through the Pd/Pt circuit was changed to a new steady-state value. In response to this perturbation in the steady-state condition, the power in the resistor was then adjusted to maintain the cell temperature at *T*_{cell}. The ratio between the power input into the Pd/Pt circuit and the power input in the resistive load then allowed accurate determination of the steady-state power production from the Pd/Pt electrolysis circuit. Experiments were performed for both increases and decreases in the current density of the Pd/Pt system. An additional calibration of the rate of heat loss from the dewar was obtained by varying the resistor power at a constant Pd/Pt current density. This latter method was used to calculate the heat balance for the Pd/0.1M LiOH-H₂O/Pt control cell.

Table 3 summarizes the results of these calorimetry experiments. Cells operated in H₂O or D₂O electrolytes showed less heat produced in the electrolyte than power input into the Pd/Pt circuit. This is consistent with the expectation for an electrolysis reaction in which some of the input power will be carried away in the enthalpy of the D₂ (or H₂) and O₂ gases escaping from the measurement system. A correction for the enthalpy contained in the escaped D₂ and O₂ gases can be conveniently performed by subtracting 1.54 V for D₂O (1.48 for H₂O) from the applied voltage¹⁵; *V*' = *V* - 1.54 is therefore the amount of voltage that will be effective in heating the contents of the D₂O electrolysis cell¹⁶. Applying this correction, we obtained agreement between power input in the Pd/Pt circuit and power production in the electrolysis cell to better than 6%, which is in excellent agree-

ment with expectations based on conventional chemical reaction processes for the electrolysis of water. The small excess power production over the expected lower limit is in accord with previous studies that have identified a current-density-dependent H₂-O₂ recombination process as the cause of some heat production above the value expected in the absence of any recombination¹⁷.

We have also identified several subtle sources of possible error in the calorimetric measurements. If power was applied only to the load resistor until *T*_{cell} was attained and then the Pd/Pt circuit was turned on and the load resistor turned off, incorrect estimates of Pd/Pt power production were obtained from the new steady-state temperature reading. This resulted from changes in the heat losses when the Pd/Pt cell was being charged, which in turn resulted in changes of the Newton's cooling constant, as seen in Fig. 3. Errors of ±50% in the Tronac, Inc. vacuum-jacketed calorimeter dewar could be obtained in this fashion, whereas smaller errors were observed in the air-jacketed dewar. We also sometimes observed abrupt or gradual changes in the rate of heat loss from these cells, presumably resulting from a change in the rate and/or form of gas evolution¹⁸. These changes often resulted in a sustained temperature rise of the cell (which might be interpreted in terms of the onset of excess enthalpy production), but recalibration with the load-resistor method during this period showed no evidence for any anomalous power production, even after the reported activation period for the Pd rods^{1,19} had been exceeded.

We also observed that even in the presence of vigorous evolution of gas bubbles at current densities >100 mA cm⁻², temperature gradients existed in cells that did not have efficient mechanical stirring. With power applied simultaneously to the resistive load and the Pd/Pt circuit, we found a temperature differential of up to 2 °C in an unstirred operating cell (6–8 °C above ambient) of dimensions very similar to those used in

previous work¹. This differential exists because of inadequate mixing of the various sources of heat in the cell, including the heat of neutralization at the anode, the resistive heating in the cathode-anode gap and the heating from the calibration load resistor. This error could only be eliminated by efficient mechanical stirring of the cell; thus, calorimetric measurements and calibrations performed in unstirred solutions, even in the presence of gas evolution, may result in errors in enthalpy measurements. We have also observed that the apparent Newton's cooling constant for the above cell was a complicated function of the current density applied to the Pd cathode (Fig. 3) and of the position of the thermistor relative to the resistor; failure to account for changes of this constant led to systematic errors which resulted in errors in the amount of power produced by the electrolysis circuit. We note that, without a total heat-flow balance in each electrolysis cell, the observation of a small temperature differential between a Pd/D₂O cell and a Pd/H₂O cell (A. Belzner, U. B. Bischler, S. Crouch-Baker, T. M. Gur, G. Lucier, M. Schreiber and R. A. Huggins, presented at the 175th meeting of the Electrochemical Society, 8 May 1989, Los Angeles) is complicated by the known differences in heat capacity, thermal conductivity, masses of the evolved gases, electrical conductivity and resistivity of Pd rods between the light-water and heavy-water solutions. These complications make interpretation of differential calorimetric studies of the Pd/H and Pd/D system extremely difficult, and these factors must be accounted for in a quantitative and rigorous fashion before any unusual enthalpy production can be documented.

Minimum voltage requirements for electrolysis

We have also investigated the minimum voltage that is required in a regenerative-cell arrangement to maintain a 64 mA cm⁻² current density through a Pd rod. This voltage would be obtained when the anode was performing the reverse chemical reaction of the cathode; that is, when the anode is 'depolarized' by introduction of D₂(g). In this situation, the anode could exclusively perform the oxidation of D₂(g) → 2D⁺(aq) + 2e⁻ (as opposed to the oxidation of D₂O to O₂(g)), and this would result in a lower cell voltage at constant cathode current density. At 64 mA cm⁻² on a 0.22 × 10-cm rod with a Pt-foil counter electrode of 30-cm² area, our cell voltages are close to that reported in previous work¹, but we have been unable to observe cell voltages <3.8 V even in the presence of a vigorous flow of 1 atm of D₂(g). We attribute this to the relatively low solubility of D₂(g) in D₂O and to the high cell resistance (30–50 Ω) in the 0.1M LiOD electrolyte in the present cell configuration. Also, steady-state current-voltage measurements relative to a Pd/D₂ reference electrode indicated a resistance-corrected potential difference of -0.8 V during cathodic charging of the Pd at current densities of 60 mA cm⁻², which is in accord with previous determinations of this potential drop¹. Thus, the minimum potential difference between a charging Pd rod and an anode at the reversible D₂(g) → 2D⁺(aq) + 2e⁻ potential must be >0.8 V and is more typically 3–4 V because of contributions from cell resist-

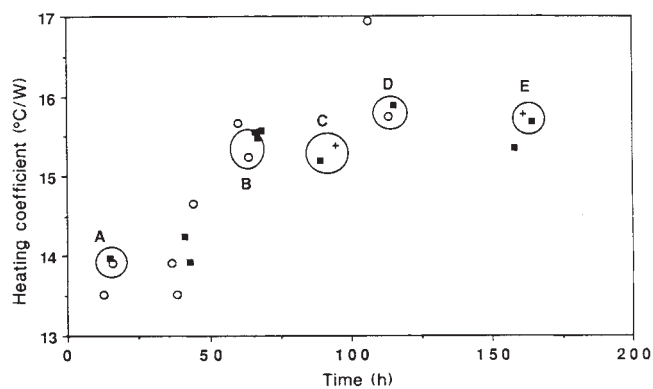


FIG. 3 Heating coefficient plotted against time determined using an isoperibolic calorimetry cell with a Pd/D cathode and a Pt anode in 0.1M LiOD/D₂O. The cathode was a cast Pd rod, 0.21-cm diameter × 2.1-cm length. The letters refer to the data pairs given in Table 3. Symbols refer to the following current densities through the Pd/Pt circuit: ○ = 72–74 mA cm⁻²; ■ = 108–115 mA cm⁻²; + = 140 mA cm⁻².

ance in aqueous 0.1M LiOD. Also, our measurements show that the minimum open-circuit potential difference between a charged Pd rod and a Pd α/β reference electrode is <-0.6 V. Based on these measurements, we conclude that the output/input power ratios >100% reported previously based on an assumed 0.5-V cell voltage¹ are strictly hypothetical quantities. We also conclude that this 0.5-V value is inaccessible on both thermodynamic and practical (resistive loss) grounds, and recommend that any excess power generation rates calculated using this hypothetical voltage be interpreted with these caveats in mind.

Conclusions

Within strict error limits, a variety of Pd samples have displayed no anomalous behaviour in the production of excess enthalpy, neutrons, γ-rays, tritium or helium. We have also identified several possible sources of error in isoperibolic calorimetry experiments that might account for excess power production reported in other work. Of course, we cannot exhaust all of the possible variations in heat treatment, impurity content of Pd and D₂O, and so on that might potentially differentiate our procedures from those performed in other laboratories. We note that the present work is not directly relevant to the studies of Jones *et al.*²⁰ because of differences in the composition of the electrodes and electrolyte. At this point however, evidence supporting a high rate of fusion reactions in Pd that is electrochemically charged with D₂, especially without concomitant radiation production, will clearly require a complete documentation of the methods, the state of the Pd and the error limits in the experiments, as well as a complete explanation of the differences in method between this work and other studies. □

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The *glass* gene encodes a zinc-finger protein required by *Drosophila* photoreceptor cells

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Null mutations of *glass* specifically remove photoreceptor cells, leaving other cell types intact. We have isolated the *glass* gene and have shown that its transcript encodes a putative protein of 604 amino acids with five zinc-fingers. The *glass* product may be a transcription factor required for the development of a single neuronal cell type.

THE *Drosophila* visual system uses specialized sensory neurons as photoreceptor cells. They occur in three organs in *Drosophila*: the larval eyes¹ (Bolwig organs), the adult ocelli² and compound eyes³. Like all peripheral neurons, photoreceptor cells extend axons and express neural antigens such as those detected by the monoclonal antibody 22C10 (ref. 4) and anti-horseradish peroxidase antibody (HRP) (ref. 5). In addition, photoreceptor cells express a number of cell-type specific proteins such as rhodopsins⁶, chaoptin⁷ and the *ninaC*⁸ and *trp*⁹ gene products.

A number of mutations have been identified that affect the development of the *Drosophila* visual system, and of these *glass* is unusual in that it affects all photoreceptor cells. The first *glass* mutation was identified by Muller in 1918 (ref. 10). In *glass* mutants the compound eyes are reduced in size, the regular array of facets (or ommatidia) is disrupted, and the eye partially lacks pigment (Fig. 1*a, b*). The ocelli are white, flattened and bear small protrusions¹⁰. Internally, the photoreceptor cells of the compound eyes are missing and the optic lobes of the brain are degenerate. Meyerowitz and Kankel¹¹ used somatic mosaics to show that this brain phenotype depends exclusively on the retinal genotype. Ready and his co-workers¹² reported that with a weak *glass* allele, *gl*³, the photoreceptor cells of the compound eye degenerate and die in the mid-pupal stage, at the time when such cells would normally be producing their photosensitive organelles (the rhabdomeres). They also reported that at a time when the photoreceptor cells are still present in the third-instar imaginal disc, there is no expression of an antigen (detected by the monoclonal antibody 301) specific to photoreceptor cells.

Here we show that null alleles of *glass* are recessive and that mutants homozygous for these alleles are viable. We also describe the early effects of these alleles on the precursors of photoreceptor cells in the eye imaginal disc. Immunohistochemistry shows that in *glass* mutants these cells express neural antigens but not an antigen specific to photoreceptor cells. Mosaic analysis showed that in the compound eye, *glass* function is autonomously required for the development of the photoreceptor cells exclusively. Similarly the photoreceptor cells of the ocelli and Bolwig organs require *glass* function. Cytogenetic analysis revealed that the *glass* gene lies in polytene band 91A1-2, on the right arm of the third chromosome. We have isolated the *glass* gene and characterized its transcript, which can encode a 604 amino-acid protein with five zinc-finger domains, similar to those found in known transcription factors¹³⁻¹⁵. Thus, the *glass* gene product may be a transcription factor required for gene expression specific to photoreceptor cells.

Null mutants for *glass* are viable

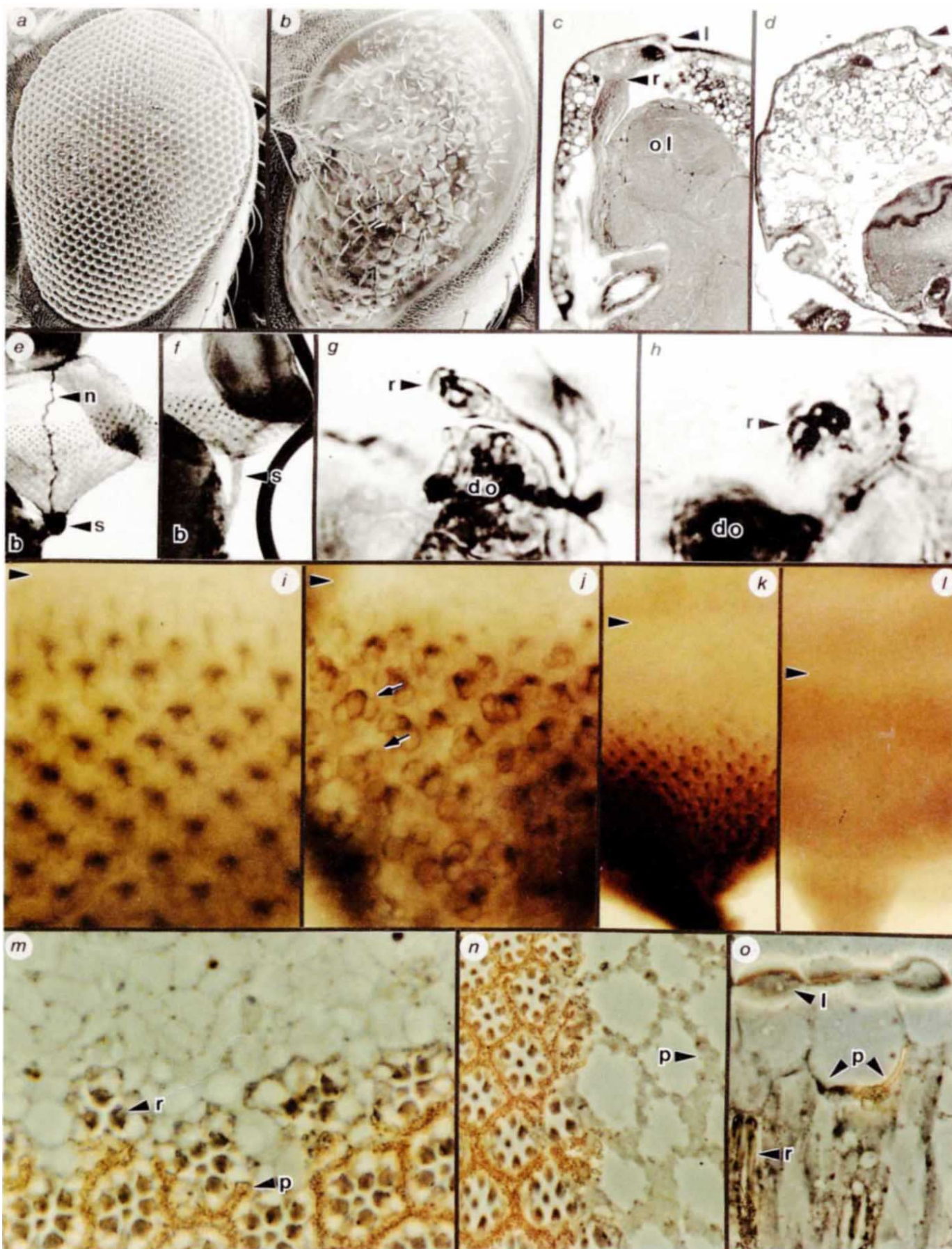
If *glass* has a general role in development then we might expect true null alleles to be recessive lethals. There are two classes of

glass alleles: strong alleles (Fig. 1*b*), causing part of the eye field to lack pigment; and weak alleles, producing fully pigmented eyes. These also differ in the degree of the morphological disorder. Of the five previously described *glass* alleles (four spontaneous point mutations and one translocation), four are strong and one, *gl*³, is weak; all are recessive and not lethal when homozygous. When placed in *trans* configuration with a deletion Df(3R)P14 the strong alleles remain strong, whereas *gl*³ becomes stronger. As a deletion is not lethal when placed in *trans* to any of the existing alleles, null alleles can be recovered by screening for mutations that fail to complement an existing allele. We recovered 24 new alleles in this way, using chemical mutagenesis with EMS (ethylmethane sulphonate). Four of these are weak and twenty are strong. All were tested for their effect on viability in *trans* to a large deletion for the region Df(3R)P14; 23 were recessive and not lethal. The remaining allele, *gl*^{B16}, was a recessive lethal, but has been shown subsequently to be a deletion of ~20 kilobases (kb), which removes at least one additional transcription unit (Fig. 2). We conclude that *glass* is not required for *Drosophila* viability and that the strong *glass* alleles are likely to be null.

The mutant phenotype

We examined the effects of *glass* mutations on the development of all three visual organs in *Drosophila*. In the larva, each of the Bolwig organs consist of 12 cells that arise in mid-embryogenesis, and come to lie on either side of the mouth hooks¹⁶. The axons of the photoreceptor cells form a fasciculated nerve that extends across the face of the eye imaginal disc and down the optic stalk into the optic lobes. In the strong allele *gl*² the Bolwig nerves are absent in the third larval instar (Fig. 1*e, f*). They are present, however, in larvae of the weak allele, *gl*³. Neither *gl*³ nor *gl*² mutants express chaoptin⁷ (data not shown), a protein specific to photoreceptor cells and seen in these cells in the wild-type. The normal Bolwig organ is absent from *gl*² embryos, and in its place is a loosely-clustered group of abnormal neurons, with random axonal projections (Fig. 1*g, h*). Mutants for *glass* have ocelli which are flattened and lack pigment¹⁰. We examined sections of *glass*- and wild-type heads (Fig. 1*c, d*) and found that the entire ocellar neuropil is absent from the mutant. By analogy to the optic lobes¹¹, the loss of the ocellar neuropil may be a secondary consequence of the loss of the ocellar photoreceptor cells.

The comprehensive description of the development of the compound eyes permits a deeper analysis of the role of *glass* in their development than for the other visual organs. The photoreceptor cells of the compound eyes are recruited from the cells of the eye-antennal imaginal disc in the late third instar without reference to their lineage¹⁷. Differentiation does not occur synchronously, but progresses across the disc from posterior to anterior. An indentation in the disc, the 'morphogenetic furrow'¹⁸ is coincident with the onset of neural differentiation. Cells are sequentially recruited into the assembling clusters, beginning with the 8 photoreceptor cells and then 12 accessory cells, to produce each of the 800 simple eyes or 'ommatidia' that comprise the compound eye¹⁹. In wild-type eye-imaginal discs the developing photoreceptor cells express certain antigens in a specific sequence²⁰. Even in null *glass* mutants cells emerge from the morphogenetic furrow, form clusters similar to those of wild-type discs, express neural antigens detected by the anti-



body 22C10 (Fig. 1*i, j*) and anti-HRP antibody (data not shown), and extend axons which grow down the optic stalk towards the optic lobes. Eye discs of *glass* mutants, however, display three developmental defects from a very early stage: there are errors of spacing, of orientation and of cell number in the assembling ommatidia. Moreover, there is no detectable chaoptin⁷ expression, even within the weak allele, *gl*³ (Fig. 1*k, l*), although the photoreceptor-cell precursors are viable at this time and are expressing general neural antigens. This is similar to the block in expression of the antigen recognized by the antibody 301 reported previously¹². In wild type flies the *sevenless* protein is expressed in the cone as well as the photoreceptor cells²¹. Even in the strong allele *gl*², *sevenless* expression resembles that of wild type as closely as can be determined given the abnormal morphology of the assembling ommatidia (data not shown). Thus, *glass* function is absolutely required for the expression of chaoptin, normally specific to the photoreceptor cells, in contrast to the more generally expressed antigens detected by the antibody 22C10, anti-HRP antibody, and the antibody to the *sevenless* gene product.

From this phenotypic analysis we conclude that in *glass* mutants photoreceptor-cell precursors are recruited into developing ommatidia and proceed to establish a neural identity. They appear, however, to be unable to progress to photoreceptor cell identity, and later die¹².

Cell autonomous requirement for *glass* function

To reveal which cells in the compound eye require *glass* function we produced clones of cells doubly mutant for *glass* and the cell-autonomous eye-colour gene *white* (*w*) in otherwise wild type eyes²². In one experiment five *gl*³ clones were analysed. Although *w*⁻ (and therefore *glass* mutant) pigment cells could be seen (Fig. 1*m, o*), there were no mutant photoreceptor cells.

- FIG. 1 (Opposite) The *glass* mutant phenotype. *a, b* Scanning electron micrographs (×100) of wild-type and *gl*² *Drosophila* compound eyes. *c, d* Sagittal sections (×100) through wild-type- and *gl*² *Drosophila* heads showing the ocellar lens (l), the ocellar photoreceptor cells (r) and the optic lobes of the brain (ol). *e, f* Whole mounts of wild-type and *gl*² third-instar eye imaginal discs and brain (×1,350) stained with antibody 22C10, showing the brain (b), optic stalk (s) and Bolwig's nerve (n). Note the lack of Bolwig's nerve in *glass* flies (panel *f*). *g* (wild-type) and *h* (*gl*²) Dorsal anterior views of 8–12-hour embryos (×600) stained with antibody 22C10 showing the photoreceptor cells (r) above the dorsal organ (do). Allele *gl*² causes elimination of the normal Bolwig organ and in its place are neuronal cells (r) of aberrant morphology. *i* (wild-type), *j* (*gl*²), *k* (wild-type) and *l* (*gl*³). Whole mounts of third-larval-instar eye imaginal discs dissected to show the ommatidial preclusters. *i* and *j*, (×3,000) are stained with antibody 22C10; *k* and *l* (×1,200) are stained with anti-chaoptin antibody⁷. The morphogenetic furrow of each disc is indicated by an arrow head. In *j*, *gl*² mutant pre-clusters show errors of position, orientation and cell number. An extreme example of an error of orientation is shown by the two arrows in *j*. Allele *gl*³ deletes expression of the product of the gene encoding chaoptin, as seen by staining with antibody 24B10 (anti-chaoptin) (compare *k* and *l*). *m* (*gl*³), *n* (*gl*²) and *o* (*gl*³). Sections of mosaic compound eyes seen in phase contrast. They show the margins of *glass* clones marked with *white*. Photoreceptor and pigment cells can be scored for both *glass* and *white* genotype by the presence or absence of pigment granules. *m* and *n* are tangential sections; *o* is a radial section. Representative photoreceptor cells (r), pigment cells (p) and a lens (l) are indicated. Both the strong allele *gl*² and the weak allele *gl*³ show similar results. In no case is a *glass*-mutant photoreceptor cell seen, whereas many *glass*-mutant pigment cells are observed.

METHODS. Scanning electron micrographs were prepared from gold-coated whole mounts. Head sections (*c, d*) were fixed and sectioned as previously described⁴⁰, stained with toluidine blue and photographed with bright-field illumination. Embryos (*g, h*) were prepared as described previously⁴¹, stained with antibody 22C10, visualized with HRP-linked secondary antibody and photographed with bright-field illumination. Eye imaginal discs (*e, f, i, j, k, l*) were prepared as previously described^{20,21} and stained with antibody 22C10 or anti-chaoptin as above, visualized with HRP-linked secondary antibody and photographed with bright-field illumination. The mosaic clones seen in *m, n* and *o* were induced in larvae of the genotype *w*¹¹¹⁸, P(*w,ry*)D[3*gl*^{+/gl}] as described previously²².

In 114 mosaic ommatidia with reduced numbers of photoreceptor cells, 694 *w*⁺ and no *w*⁻ photoreceptor cells were scored. A second experiment with the strong allele *gl*² produced similar results (Fig. 1*n*). As all *glass*-mutant eyes retain some lenses and bristles (Fig. 1*b*), the cone- and hair-nerve-group cells cannot have an absolute requirement for *glass* function. We conclude that only the photoreceptor cells have an absolute and cell-autonomous requirement for *glass* function in the

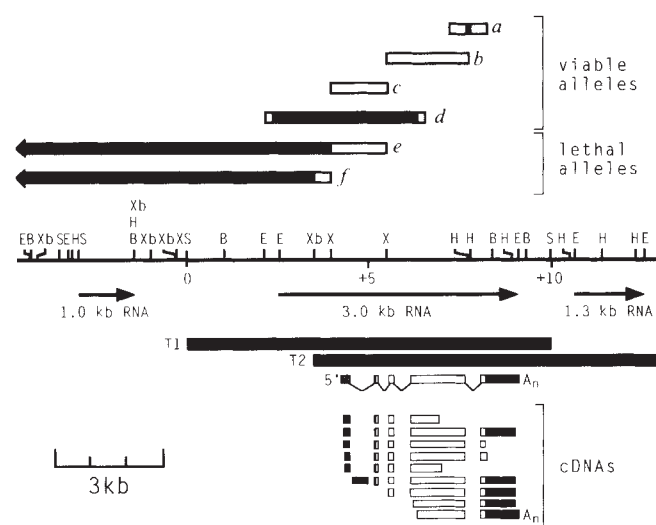


FIG. 2 The structure of the *glass* gene. The central restriction map is that of λ -5F2, a wild-type *glass* genomic clone. Restriction sites are shown for *Bam*HI (B), *Eco*RI (E), *Hind*III (H), *Sal*I (S), *Xba*I (Xb) and *Xho*I (X). The coordinates below the map are in kilobases from a *Sal*I site at position 0. The centromere is to the right and the telomere to the left. All the DNA in this map lies within polytene band 91A1-2. Above the map, intervals affected by some *glass* alleles are shown. The γ -ray-induced allele, *gl*^{WGI} deletes ~600 base pairs (bp) within the limits shown by the open block *a*, and must affect the included filled block as it deletes the two *Hind*III sites at +7,678 and +7,754. Block *b* is a 2,333 bp interval between the *Xho*I site at +5,345 and the *Hind*III site at +7,678. The spontaneous allele *gl*¹ affects *b* in a complex manner, the spontaneous allele *gl*² deletes ~1 kilobase (kb) from *b*, and both the spontaneous translocation T(2;3)*gl*^{63d29} and the X-ray-induced inversion In(3R)*gl*^{BX6} break within *b*. Block *c* is a 1,395 base interval between the *Xho*I sites at +3,950 and +5,345. The spontaneous weak allele *gl*³ inserts about 2.5 kb into *c* and the spontaneous allele *gl*^{60j} inserts more than 30 kb into *c*. Block *d* shows the extent of the DNA affected by the X-ray-deletion allele *gl*^{BX5} (~4.5 kb). Similarly blocks *e* and *f* denote the DNA affected by the lethal EMS-induced allele *gl*^{B16} and X-ray-induced allele *gl*^{BX7} (which remove ~20 and 18.5 kb respectively). The open portions of the blocks indicate the limits of the uncertainty in mapping the endpoints of the deletions, and the included filled blocks indicate the DNA that must be deleted. Immediately below the map three arrows indicate probes that detect three transcripts on RNA blots (see Fig. 4); the orientations and sizes of the RNAs are indicated. The shaded blocks *T1* and *T2* show fragments inserted into the genomes of *glass* mutant flies by P-mediated transformation. *T1* is a 9,954 base *Sal*I fragment which rescues *glass* gene function (see Fig. 3), and has been sequenced (see Fig. 5*a*). *T2* is a 9.5-kb *Xba*I fragment that does not rescue *glass* function. The structure of the *glass* transcript is shown in detail below *T1* and *T2*. The 5'-end was determined by RNase protection and primer extension (see Fig. 5*b, c*). The four introns were placed by alignment of the sequences of the nine cDNA clones shown with the genomic sequence. The polyadenylation site (*A_n*) was inferred from the longest 3'-cDNA (shown last) which terminates in a poly(A) tract. **METHODS.** Clone λ -5F2 was isolated from a *Sau*3A partial digestion library in the vector λ -FIX (Stratagene) by a chromosome walk from the insertion site of P(*w, ry*)A¹⁰-1 in polytene band 91B2-3. The restriction map was determined from digestion of the five *Xba*I subclones, and confirmed in the sequenced region. Mutant lesions were detected as polymorphisms on genomic DNA blots. Transcripts detected by RNA gel blots (see Fig. 4), and their orientations determined by single-stranded ³²P-labelled RNA probes made from subclones in pBlueScript KS M13+ (Stratagene). Complementary DNAs were isolated from an eye-disc cDNA library in λ gt10 (gift from A. Cowman) using various genomic probes.

compound eye. The absence of some of the accessory cells in the eyes of the null mutants may have been a secondary consequence of early photoreceptor-cell death, as accessory cells do not have an obligate requirement for *glass* function.

Isolation of the *glass* gene

As attempts to induce *glass* mutations by hybrid dysgenesis²³ were unsuccessful (no new alleles were recovered in about 380,000 F1-generation flies) we used an alternative cloning strategy employing a chromosome walk. To refine the cytological location of the *glass* gene we examined existing and newly generated chromosome aberrations within the region. The most informative mutant chromosomes showed an inversion $\text{In}(3\text{R})\text{gl}^{\text{BX}6}$ and a translocation $\text{T}(2;3)\text{gl}^{\text{63d29}}$, both of which appear to have breakpoints within the gene. Consistent with the analysis of 14 large deletions, these place *glass* in polytene band 91A1-2 (data not shown). The closest suitable starting point was the insertion $\text{P}[(w,ry)\text{A}^{\text{R}}]0-1$, a derivative of $\text{P}[(w,ry)\text{A}^{\text{R}}]4-3$ (ref. 24), which is an allele of *fruitless*²⁵ in polytene band 91B2-3 and lies 0.23 centimorgans distal to *glass*. A clone including $\text{P}[(w,ry)\text{A}^{\text{R}}]0-1$ sequences was recovered from a genomic library by use of a *white* probe, and flanking genomic DNA was used to start a walk in cosmid and phage libraries. After ~100 kilobases (kb) the breakpoint of $\text{T}(2;3)\text{gl}^{\text{63d29}}$ was crossed in clone λ -5F2 (Fig. 2). Restriction-map polymorphisms were found for nine *glass* alleles, and the lesions of eight are shown in Fig. 2. The two lethal alleles without cytologically visible chromosome aberrations (gl^{B16} —see above, and $\text{gl}^{\text{BX}7}$) were also analysed and found to delete DNA starting within λ -5F2 and extending distally into the next phage. All eleven alleles located affected a 7.3-kb *Bam*HI fragment in λ -5F2 (Fig. 2), and of these, the nine non-lethal alleles affected only this fragment.

To determine the limits of the *glass* gene we used P-mediated germline transformation²⁶. Two fragments from λ -5F2 (*T1* and *T2* in Fig. 2) were introduced into the genomes of *glass*-mutant flies. Based on external morphology, all of the *T1* lines rescued the *glass* phenotype, whereas all of the *T2* lines did so very weakly or not at all. Three of the *T1* lines were crossed into a *glass* null (gl^2) genetic background and the structure of the resulting retinas examined in section. All three were wild type

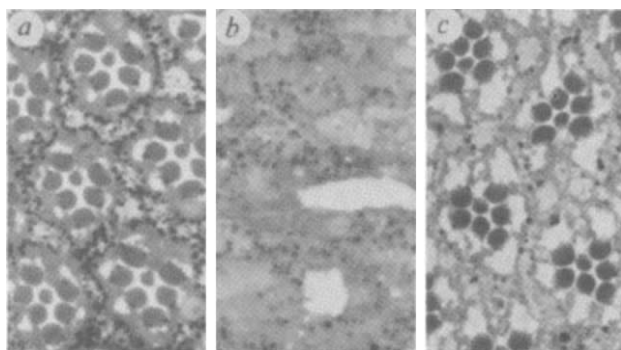


FIG. 3 Transformation rescue. Tangential sections of compound eyes of three genotypes: a, Wild-type; b, Allele gl^2 ; and c, Allele gl^2 with one copy of the 10-kb *Sal*I fragment (*T1*) inserted in polytene band 17C.

METHODS. *T1* and *T2* (see Fig. 2) were inserted into the *Not*I site of the ry^+ P-element transformation vector pCaWc (D. Bowtell, unpublished data), by the *Not*I shuttle vector pHSX (K. Jones, unpublished data). These were injected into embryos of the genotype $\text{gl}^3, \text{ry}^{\text{506}}$, and G1 transformants selected on the basis of ry^+ phenotype²⁶. *T1* rescues *glass* function whereas *T2* does not. Insertion sites were mapped to chromosomes for 33 *T1* lines and 23 *T2* lines. Six *T1* and four *T2* lines were retained and their position of insertion and copy number determined by chromosome *in situ* hybridization and genomic DNA blotting (data not shown). Three *T1* lines were crossed into a gl^2 genetic background and sections cut and stained as described in Fig. 1.

(Fig. 3), although one ommatidium (fewer than 1% of the total) was seen with nine rather than eight photoreceptor cells. Thus this 10-kb *Sal*I fragment (*T1*) contains all the DNA sequences necessary and sufficient for *glass*-gene function.

Gene structure and expression

A probe from within the transforming fragment *T1* was found to hybridize to a 3.0-kb poly(A)⁺ RNA in adult heads (Fig. 2). Outside *T1* lie flanking transcription units (Fig. 2). Analysis of poly(A)⁺ RNA blots showed that in all 12 non-lethal mutations examined, the 3.0-kb RNA alters in size or abundance; indeed, most alleles appear to be transcript nulls (Fig. 4). This could be simply explained if *glass* expression were specific to photoreceptor cells in the adult, as these cells are absent at this time in *glass* mutants. None of the 12 alleles affect the flanking transcripts (Fig. 4). As the transforming fragment *T1* appears to encode only the 3.0-kb messenger RNA, and all the *glass* alleles tested affect only this transcript, we conclude that it is the *glass* mRNA.

Poly(A)⁺ RNA prepared from several stages of the *Drosophila* life cycle (Fig. 4) showed that the 3.0-kb mRNA is first detected

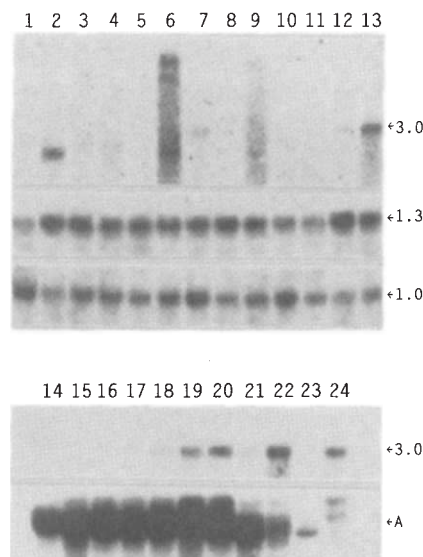


FIG. 4 RNA blot analysis. Lanes 1–13 show a blot of total adult poly(A)⁺ RNA (2 µg per lane) prepared from different *glass* alleles and hybridized sequentially to the three probes shown in Fig. 2. The 3.0-kb RNA is the *glass* transcript, and the 1.3- and 1.0-kb RNAs are the left- and right-flanking transcripts. The lanes are: 1, gl^1 ; 2, gl^2 ; 3, gl^3 ; 4, gl^{60J} ; 5, $\text{T}(2;3)\text{gl}^{\text{63d29}}$; 6, $\text{In}(3\text{R})\text{gl}^{\text{BX}6}/\text{TM3}\text{gl}^{\text{B2}}$; 7, $\text{gl}^{\text{BX}2}$; 8, $\text{gl}^{\text{BX}5}$; 9, $\text{gl}^{\text{BX}9}$; 10, gl^{WG1} ; 11, gl^{WG2} ; 12, gl^{WG3} ; and 13, gl^+ (Canton-S). All 12 *glass* mutations affect either the size or the abundance of the 3.0-kb transcript, but none affect either of the flanking RNAs. Lanes 14–24 were loaded with poly(A)⁺ RNA (5 µg) prepared from different life cycle stages, hybridized to a probe for the 3.0-kb *glass* transcript, and then re-hybridized with a probe for actin (A) transcripts. The lanes are: 14, 0–24 hour embryos; 15, first instar larvae; 16, second instar larvae; 17 and 18, early- and late-third instar larvae; 19, 20 and 21, early, mid and late pupae; 22 and 23, heads and bodies respectively, from three-day-old adults; and 24, total third instar larval imaginal discs (gift of J. Fristrom⁴²).

METHODS. RNA was prepared from tissue ground in liquid nitrogen and dispersed in ten volumes of a solution of Tris buffer (0.1 M, pH 8.0), EDTA (10 mM), NaCl (0.35 M), SDS (2%) and Urea (7M). This extract was immediately and repeatedly extracted with an equal volume of 1:1 phenol:chloroform and then precipitated with ethanol. The resulting nucleic-acids pellet was resuspended in diethyl pyrocarbonate (DEPC)-treated NaCl (0.1M) and reprecipitated, washed with ethanol (70%) and resuspended in DEPC-treated water. Poly(A)⁺ RNA was prepared by oligo(dT) cellulose affinity chromatography and was electrophoresed through formaldehyde/agarose denaturing gels and transferred to Hybond-N (Amersham). The blots were hybridized to ³²P-labelled nick-translated DNA probes.

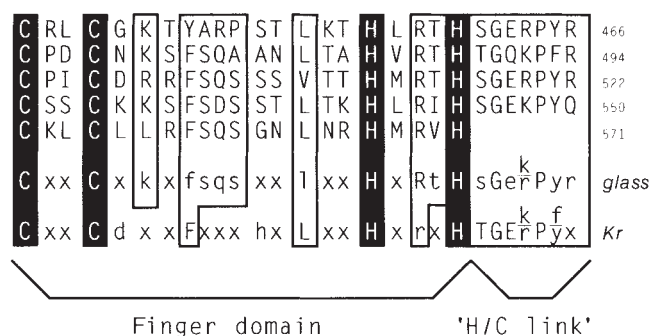


FIG. 6 Zinc-finger domain. The putative *glass* protein sequence in the single-letter code from residue 439 to 571 is displayed as five 28-residue repeats. A consensus is drawn with a capital letter indicating a residue found in all repeats, and a lower-case letter indicating a residue found in at least three of the repeats. The *glass* consensus is compared with that of the *Krüppel* (Kr) finger domain. The invariant C and H residues are highlighted.

that matches the consensus for *Drosophila* transcription start sites in six out of seven nucleotides³⁰.

The *glass* protein has zinc-fingers

The long open reading frame of the 3.0-kb RNA encodes a protein of 604 amino acids that includes five zinc-finger repeats similar to those seen in the *Krüppel* protein³¹ (Fig. 6). There is a match to the 'H/C-link'³² between the fingers of five out of six amino acids. There are sequences shared between the 'finger tips' of the *glass* gene product (in this case Ser-Gln-Ser) that are not conserved in other zinc-finger proteins. DNA-sequence-binding specificity may reside in these peptides¹⁵.

Conclusions

We have shown that the *glass* gene is essential for the correct development of photoreceptor cells in *Drosophila* in all three organs in which they occur and that no other cell type in the compound eye has an obligate *glass* requirement. Our genetic and molecular data strongly support the conclusion that mutants for null alleles of *glass* are fully viable. The *glass* gene is included in a 10-kb *SalI* fragment that directs the transcription of a single mRNA of 3.0 kb. This transcript is affected by all 12 *glass* mutations examined, and 11 *glass* alleles were found to disrupt

the DNA that encodes it. These data establish that the 3.0-kb mRNA is the *glass* transcript. The long open reading frame in this RNA encodes a protein product with five zinc-finger repeats.

The post-mitotic cells that will become the photoreceptor cells of the compound eye are recruited sequentially into clusters that will later become ommatidia^{18,20}. The cells are recruited by a system of positional information thought to rely on cell contact¹⁹. Almost as soon as they become morphologically distinct they express neural antigens²⁰ and begin to extend axons. We propose that at about the time when wild-type pre-photoreceptor cells acquire neural characteristics they progress to a photoreceptor-cell-type identity and that this specification requires *glass* function. The evidence for this early role for *glass* is found in the early disc defects. Thus *glass* may act continuously throughout the life of these cells, beginning at the earliest stages of their differentiation, to induce photoreceptor-cell-type specific gene expression.

The *glass* protein may directly regulate transcription. It has five zinc-finger repeats similar to those found in the *Krüppel* gene product, and such proteins can bind DNA, such as SP1¹⁴, the product of *Krüppel*³³ and TFIIA¹³. Two zinc-finger proteins are known to be activators of transcription *in vitro* (TFIIA¹³ and SP1¹⁴), and some have been implicated genetically as regulating the expression of other genes (the product of *Krüppel*³⁴, SW15³⁵ and ADR1³⁶). In the adult, *glass* may be expressed solely in the photoreceptor cells, as the RNA blots of adult *glass* mutants showed that most *glass* alleles lack transcripts. Several zinc-finger proteins have been shown to be tissue or regionally restricted (the product of *Krüppel*³⁷ and *Krox-20*³⁸, and GLI³⁹). However, the protein encoded by *glass* is one of the few putative cell-type specific transcription factors described.

In the absence of *glass* function the pre-photoreceptor cells in the eye disc proceed to a neural identity but fail to become photoreceptor cells. They show early defects, perhaps due to specific failures of gene expression early in photoreceptor cell development. They are not transformed into any other cell type, but die¹². Possibly the photoreceptor cells become irrevocably committed to their cell fate independent of *glass* function but cannot execute this developmental pathway without *glass* function. The *glass* mutant phenotypes of the Bolwig organs and the ocelli are consistent with a similar role for *glass* function in their photoreceptor cells. The mutant phenotype, genetics and molecular biology of the *glass* gene imply an early and central role for *glass* in the differentiation, and possibly the determination, of photoreceptor cells in *Drosophila*. □

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Large-scale magnetic-field structure in the spiral galaxy M83

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THE large-scale structure of magnetic fields in spiral galaxies, as deduced from the linear polarization of scattered starlight¹ and from the intensity and linear polarization of continuum synchrotron radio emission², tends to follow the visible spiral arm structure. Both axisymmetric (in the galaxies IC342 and M31; refs 3 and 4) and bisymmetric (in M81; ref. 5) patterns are seen, the latter showing a field reversal between the arms. We have measured the linear polarization at a spatial resolution of ~ 2 kpc in VLA (Very Large Array) observations of 20-cm continuum radio emission from the bright, nearly face-on, spiral galaxy M83 (NGC5236). The strongest linearly polarized emission is from two giant arcs, ~ 30 kpc long, situated roughly opposite each other in the dark outer regions of the galaxy, ~ 12 kpc from the centre. These regions do not coincide with any prominent spiral arm tracers, and from a comparison with earlier results at 6-cm wavelength, we conclude that the low polarization in the centre of the galaxy indicates disorder in the interstellar magnetic field, probably connected with star formation activity observed there.

The effects of Faraday rotation on the plane of polarization can be determined from observations in two or more widely separated frequency bands. These effects are also minimized for galaxies that are orientated approximately 'face on', in which the total path length through the galaxy is shortest and the component of the magnetic field that is parallel to the line of sight is probably less significant. The southern galaxy M83 has a low inclination⁶ of $\sim 24^\circ$ and is therefore very suitable for polarization studies. It is also bright both at optical and radio wavelengths. The distance to the galaxy has been variously estimated as 3.7 Mpc (ref. 7) and 8.9 Mpc (ref. 8). We adopt the latter value, in which case 1 arcmin corresponds to 2.6 kpc.

The first high-resolution radio-continuum observations of M83 made with VLA showed faint linearly polarized emission at a wavelength of 6 cm in the inner regions of the bar⁹. Because of inadequate spatial frequency coverage, however, extended regions of low surface brightness in the disk of the galaxy were not detected. The large-scale magnetic-field structure in M83 was first measured at a wavelength of 6.3 cm with the Effelsberg 100-m radio telescope (resolution 2.5 arcmin = 6.5 kpc)¹⁰; evidence that the magnetic-field direction is roughly parallel with the spiral arms of the galaxy was found. The regions of strongest polarization did not coincide, however, with the bright spiral-arm tracers; the distribution of polarized radio-continuum surface brightness showed a depression in the inner regions of the galaxy, which was ascribed partly to rapid changes in the position angle of the (presumed strong) linear polarization over the telescope beam.

We have used the VLA in the compact 'D' configuration to observe M83 at a wavelength of 20 cm. During August 1988, the galaxy was observed for ~ 5.5 h in two 25-MHz frequency bands centred around 1,452 and 1,502 MHz. The observations were sensitive to structures as large as 30 arcmin. The data in each frequency band were analysed separately, allowing us to check the consistency of the total intensity and polarization maps. The two bands agreed very well with each other and were therefore combined. The r.m.s. noise levels in the total intensity and the linearly polarized intensity maps are ~ 120 and ~ 60 μ Jy per beam, respectively. The angular resolution of the final images is 70×30 arcsec (at position angle (PA) = 0°) corresponding to 3.0×1.3 kpc at an assumed distance of 8.9 Mpc. These maps are

shown as contour diagrams with levels increasing by factors of $2^{(N/2)}$ ($N = 1, 2, 3, \dots$) in Fig. 1a, b. In Fig. 1b, which is overlaid on an optical image of the galaxy, we have shown the direction of maximum polarized intensity (the E field orientation) as vectors, the lengths of which are proportional to the intensity of the polarized emission.

The distribution of the total intensity in Fig. 1a consists of several components. A bright nuclear source is surrounded by features that correspond to the bar and spiral arms. This structure

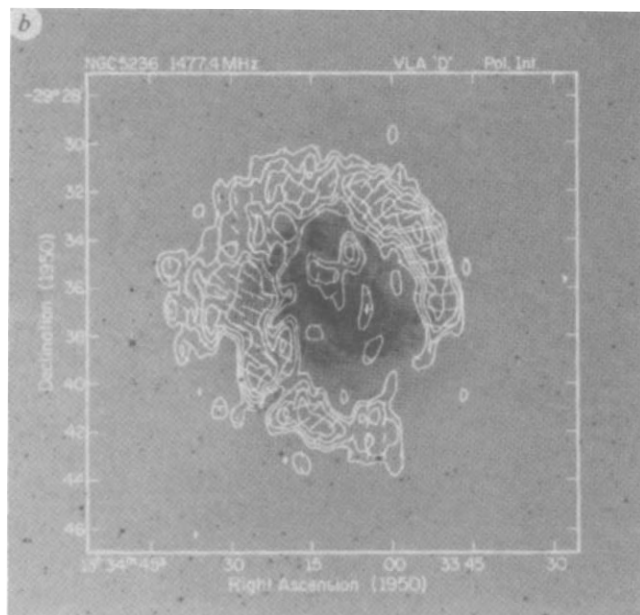
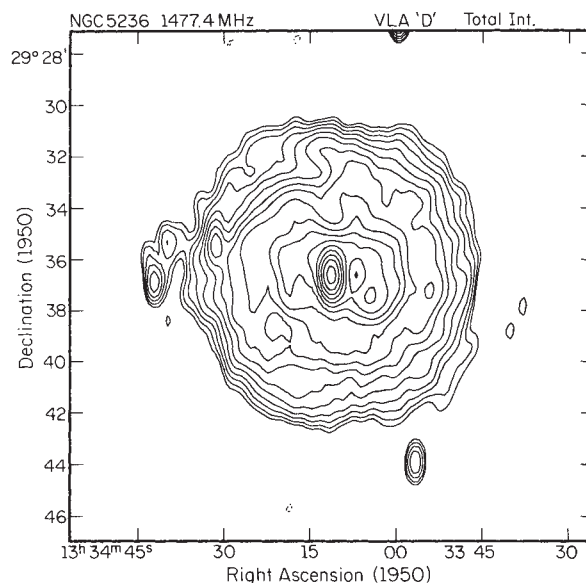


FIG. 1 a, Surface brightness of the total radio emission and b, linearly polarized radio emission from M83, observed with the VLA at a wavelength of 20 cm. Contours start at 1.0 mJy per beam (0.3 mJy per beam) in a (b), and increase by a factor $\sqrt{2}$ to maxima of 256 mJy per beam (1.68 mJy per beam); the r.m.s. noise is ~ 120 μ Jy per beam (60 μ Jy per beam). The restoring beam widths for both maps are 70×30 arcsec at PA = 0° . The short lines in b represent the strengths of the polarized emission and the direction of the maximum E. The Faraday rotation is generally small in this galaxy, so the magnetic-field direction is nearly perpendicular to these vectors.

in the disk decreases slowly in intensity with increasing radius, dropping to ~ 16 mJy per beam at a radius of ~ 3.5 arcmin. Beyond this radius a more rapid, approximately exponential, decrease sets in, and the intensity has decreased to 1 mJy per beam at 6 arcmin.

The morphology of the polarized emission in Fig. 1b differs considerably from that of the total intensity. The polarization is located in two large arc-shaped regions placed roughly symmetrically at ~ 12 kpc from the centre of the galaxy. These coincide with the outer regions of Fig. 1a where the total radio surface brightness of the galaxy disk begins to decrease rapidly. The maximum polarized radio surface brightness is detected in the southeastern and northwestern regions of the galaxy where the optical emission is faint and diffuse. The average amount of polarization in these outer arcs is $\sim 30\%$ of the total radio surface brightness, but at specific positions it is as high as 50% . In contrast, the average polarization over the optically bright inner regions of the galaxy is $<0.5\%$.

The polarization distribution at a wavelength of 20 cm bears a close resemblance to the earlier, lower resolution, observations at a wavelength of 6.3 cm (compare with Fig. 5 in ref. 10). The spatial distributions of both the intensity and the direction of the polarized emission are, in general, very similar at these two widely spaced wavelengths. The intrinsic-rotation measure of the galaxy is very low; at a resolution of 2.5 arcmin we have determined it to be -15 ± 3 rad m^{-2} between 6 and 12 kpc radius. As very little polarized emission is observed inside 6 kpc we cannot reliably calculate a rotation measure there. To appreciably depolarize the emission by differential Faraday rotation at a wavelength of 6 cm, the rotation measure would have to suddenly increase inside 6 kpc to well over $\pm 1,000$ rad m^{-2} . This seems unlikely and the explanation for the remarkable morphology of the polarized intensity in Fig. 1b must therefore be sought elsewhere. Krause *et al.*³ have suggested two alternative explanations for the appearance of strong linear polarization in the dark inner-arm regions of the spiral galaxy M81: either the field is less disturbed there, or the dynamo processes, which are thought to generate the magnetic field, may work more effectively. If a more effective dynamo results in stronger aligned fields, we would expect to see an enhancement of the total synchrotron emission as well. Figure 1a shows, however, that this is not the case; the arc-shaped regions in Fig. 1b do not appear as regions of enhanced total emission on Fig. 1a.

The dark inter-arm and outer regions of galaxy disks are also regions of low star-formation activity and less gas turbulence, and it is this aspect that we consider to be the simplest explanation of the greater degree of polarization there. From the theory of synchrotron radiation we find that the intensity of the radio emission increases with the total magnetic field, including the uniform and the randomly oriented components¹¹. The amount of linearly polarized emission, however, depends only on the uniform field. If the field is entirely uniform, the fractional degree of intrinsic polarization ρ is related to the relativistic-electron energy spectrum γ (where $\gamma = 1 - 2\alpha$ and α is the radio spectral index) according to

$$\rho = (3\gamma + 3)/(3\gamma + 7)$$

The maximum value is therefore 0.73 for a typical value of $\alpha = -0.8$, which corresponds to the spectrum of the total radio emission from M83 (ref. 10). If the field is not entirely uniform, but only a fraction f of the volume elements along the line of sight have their field oriented in the same direction (and the rest are random), then, even in the case of infinite resolution of the telescope, the degree of polarization is reduced by the factor

$$R = \left[1 + \frac{(1-f)\pi^{0.5}\Gamma((\gamma+5)/4)}{2f(\sin\theta)^{(\gamma+1)/2}\Gamma((\gamma+7)/4)} \right]^{-1}$$

where θ is the angle between the uniform component of the magnetic field and the line of sight¹². The maximum observed

value for ρ of ~ 0.5 in the polarized arcs corresponds to a degree of alignment of $f \approx 0.5$ and, with higher angular resolution, this value could increase further. In the inner optically and radio-bright regions of the galaxy, however, the average amount of polarization is <0.005 , corresponding to a degree of alignment of <0.01 .

In the regions of M83 in Fig. 1b that show intense linearly polarized radio emission, the optical image shows only very diffuse and faint spiral arm structures with thin dust lanes embedded in them, and no large H II regions of the sort found at smaller radii in the galaxy. This description also applies to M81, in which the areas of strongest polarization are to be found in the dark inter-arm regions of the galaxy⁵. IC342 also fits this picture³, although the optical spiral arms are more difficult to trace. In the bright spiral arms and inner regions of these galaxies where star formation is much more active, the polarization is low and the magnetic field is evidently tangled on length scales of 1 kpc or less. We suggest that, in these galaxies, the large-scale order in the magnetic field is being disrupted by the turbulent spatial distribution of the kinetic energy in the interstellar medium resulting from star formation. The magnetic field will dominate in regions where the magnetic-energy density $B^2/8\pi$ is larger than the turbulent energy density $\rho V_t^2/2$, where V_t is the turbulent velocity. The energy density in a 1 μ G field is comparable with the kinetic-energy density in a gas of 0.1 H atoms cm^{-3} and a turbulent velocity of 7 $km s^{-1}$. These values are in the range expected for low-density regions of the interstellar medium; if the magnetic-field strength is greater, the field can maintain a large-scale order in spite of the disruptive effects of turbulence. We therefore predict that the velocity dispersion in the interstellar gas will in general be lower in those regions of a galaxy which show a high percentage of radio-continuum polarization. Observations of the H I in NGC628 (ref. 13) indicate that the velocity dispersion of the interstellar gas is indeed greater in the spiral arms (~ 10 $km s^{-1}$) than it is between the arms and in the outer regions (typically 7 $km s^{-1}$), although we do not know the distribution of polarization in that galaxy.

As there is apparently very little Faraday rotation, the direction of the uniform component of the magnetic field is very nearly perpendicular to the direction of the E vectors shown in Fig. 1b. The magnetic-field lines are aligned in the spiral shape with pitch angles similar to those typical of the inner spiral arms, as has been found in several other spiral galaxies. Until now, this similarity has guided the theoretical work on the generation of galactic magnetic fields to consider models that involve the mechanisms which are thought to be responsible for spiral structure (see for example refs 14–16). It is worth emphasizing, however, that in the inner regions of M83, where the usual tracers of spiral structure are most prominent, there is apparently not a great deal of ordered magnetic field. The observations do show some evidence for enhanced synchrotron radio emission in the prominent dust lanes⁹, similar to the case of M51^{17,18} and modest polarization (average $\sim 8\%$), but it is clear that the magnetic field in those dust lanes is not as highly ordered as we have found in the outer regions of M83. A more detailed look at the radio-continuum properties of the inner spiral arms will require more angular resolution and sensitivity. A preliminary analysis of high-resolution (~ 10 arcsec) data that we have recently obtained with the VLA suggests that even on the scale of a few hundred parsecs the magnetic field in the inner dust lanes does not have a high degree of alignment. $\square$

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Detection of H_3^+ on Jupiter

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SINCE their detection in the high latitudes of Jupiter, first by the Voyager Ultraviolet Spectrometer (UVS) experiment^{1,2}, then by the International Ultraviolet Explorer (IUE) satellite³, the auroral particle precipitations have been associated with various phenomena in the jovian environment. In the magnetosphere, the H_3^+ ion, probably of ionospheric origin, was detected *in situ* by the Voyagers⁴. Infrared emissions were observed in spectral bands characteristic of CH_4 (ref. 5) and of other hydrocarbons^{6,7}, localized in two auroral spots^{5,8}. Here we present high-resolution spectra at a wavelength of 2 μm , in the southern auroral region of Jupiter, recorded at the Canada–France–Hawaii Telescope (CFHT), which we believe to be the first astronomical spectroscopic detection of H_3^+ . The derived rotational temperature of H_3^+ is in the range 1,000–1,200 K. Such strong H_3^+ lines could be used in future ground-based monitoring of the jovian auroral activity and to search for this molecular ion in the interstellar medium.

Observations of Jupiter were made at the CFHT with the Fourier Transform Spectrometer (FTS) during the night of 1988 September 24 (UT), between 2.0 and 2.2 μm , with a 5-arcsec aperture, at constant jovian latitude (60°S). We observed H_2 quadrupole ro-vibrational emission lines (S.J.K. *et al.*, manuscript in preparation) and determined the vibrational population of H_2 and the neutral temperature, 730 (+500/–200) K in the 0.1- μbar region. Figure 1 shows a calibrated interval of the

observed spectra in the 2- μm -wavelength window (the emission peak) which also comprise many strong emission lines. These lines are not present in the non-auroral spectrum, which is displayed for comparison. (Independent observations of some of these lines have been reported⁹ at low spectral resolution, both in north and south auroral regions.)

The strongest line, at 4,777 cm^{-1} , was monitored in individual spectra of 13-min integration each, for a total of 5 h. The emission comes from a region centred at a longitude of 60° (System III) and a latitude of 60°S. The variation of brightness with longitude (in System III), as the emitting region rotates through our stationary aperture, is reported in Table 1, along with the IUE data recorded almost simultaneously.

The IUE far-ultraviolet spectra were all taken at low dispersion with the large aperture, and the resulting H_2 spectra have been reduced and corrected for scattered light and jovian reflected sunlight using standard procedures^{3,10}. The auroral-emission brightness as a function of longitude indicates a moderately bright far-ultraviolet (FUV) aurora of 50–100 kRa (with the calibration of Table 1). The longitudinal dependence of the FUV and infrared emission brightnesses in the overlapping time period suggest a correlated process for the FUV and infrared emission. The complete reduction of the IUE observations, covering several days of September 1988, and the correlation with the mapping of the planetary disk at 7.8 μm will be published elsewhere (J.C. *et al.*, manuscript in preparation).

The rather irregular set of emission lines observed from Jupiter is reminiscent of the spectrum of a laboratory discharge through H_2 gas, in which the majority of lines belong to transitions between excited electronic states of the H_2 molecule^{11–13}. Comparisons with such spectra using various discharge sources showed, however, a significant number of coincidences for only one source, the cell used in ref. 14. This cell can operate at gas pressures up to ~50 torr and currents up to ~2.5 A. The pressure dependence of the intensities is used as a means of discriminating between the emission lines of the different species present in the plasma. Spectra taken at 10 and 50 torr showed that the relative intensities of the lines coincident with Jupiter lines generally increased with pressure, behaving in a similar way to the ro-vibrational lines of H_3^+ (ref. 14) or the ro-electronic lines of neutral H_3 (ref. 15).

The 2- μm spectrum of this discharge cell was originally recorded in 1985 in a search for the $2\nu_2$ band of H_3^+ , which is predicted to be stronger in emission per molecule than the ν_2

TABLE 1 Comparison of infrared emission in H_3^+ (4,777 cm^{-1} line) and IUE H_2 (Lyman and Werner bands) from the southern hemisphere of Jupiter during the night of 1988 September 24

Jupiter longitude* (deg)	CFHT/FTS infrared intensity† (kRa)	IUE‡ image number	IUE ultraviolet intensity§ (kRa)
210–260	—	34,301	27
245–295	—	34,302	30
280–330	8.1	34,303	35
315–5	11.8	34,304	82
350–40	15.6	34,305	102
25–75	21.1	—	—
60–110	22.8	—	—

The IUE emission (recorded 1 h 15 min after the FTS data), has been scaled to the same aperture as the FTS observations, assuming an extended emission within a 5-arcsec disk. The intensities of the jovian lines were estimated from the calibrated spectrum (Fig. 1). The 1σ level is 4 kRa for the FTS observations (1 Rayleigh = 10^6 photons $\text{cm}^{-2} \text{s}^{-1}$).

* System III

† 4 μm , 4,777 cm^{-1} H^+ line.

‡ Short Wavelength Primary camera.

§ 4 μm , 1,230–1,620 Å, H_2 band.

TABLE 2 New emission lines in the spectrum of Jupiter

	Wavenumber Jupiter spectrum (cm ⁻¹)	Intensity (Jupiter) (W cm ⁻² sr ⁻¹) (×10 ⁻¹¹)	Wavenumber laboratory spectrum (cm ⁻¹)	Molecule	Wavenumber <i>ab initio</i> calculation (cm ⁻¹)	<i>J'</i>	<i>G'</i>	Assignment <i>U'</i>	<i>J''</i>	<i>K''</i>	Einstein coefficient <i>A_{ij}</i> (s ⁻¹)
1	4,497.841	1.81	4,497.8391	H ₂				q-S ₁ (0)			
2	4,554.238	3.56									
3	4,557.057	1.89		H ₃ ⁺	4,556.883	4	6	+2	4	3	o 35
4	4,586.282	2.64									
5	4,605.653	1.83									
6	4,638.361	5.26	4,638.338	H ₃ ⁺	4,638.688	10	12	+2	9	9	o 171
7	4,649.213	3.50									
8	4,664.274	3.29	4,664.303	H ₃ ⁺	4,663.949	2	3	+2	3	0	o 65
9	4,677.268	2.18	4,677.285	H ₃ ⁺	4,677.032	3	5	+2	3	2	p 43
10	4,685.582	4.30	4,685.558	H ₃ ⁺	4,685.694	9	11	+2	8	8	p 164
11	4,712.334	—	4,712.309	H ₃ ⁺	4,711.921	5	5	+2	5	2	p 68
12	4,712.882	5.31	4,712.9054	H ₂				q-S ₁ (1)			
13	4,732.050	4.63	4,732.053	H ₃ ⁺	4,732.158	8	10	+2	7	7	p 158
14	4,777.215	12.20	4,777.228	H ₃ ⁺	4,777.123	7	9	+2	6	6	o 151
15	4,795.018	2.10		H ₃ ⁺	4,794.697	2	4	+2	2	1	p 56
16	4,804.463	3.46	4,804.406	H ₃ ⁺	4,804.080	3	4	+2	3	1	p 70
17	4,816.415	3.02	4,816.361	H ₃ ⁺	4,815.465	4	0	-2	5	3	o 89
18	4,820.542	3.90	4,820.616	H ₃ ⁺	4,820.503	6	8	+2	5	5	p 144
19	4,861.839	—	4,861.849	H ₃ ⁺	4,861.606	5	7	+2	4	4	p 136
20	4,862.385	—									
21	4,876.912	5.60	4,876.99 sh	H ₃ ⁺	4,876.231	2	1	-2	3	2	p 98
22	4,895.498	6.56	4,895.520	H ₃ ⁺	4,895.237	8	9	+2	7	6	o 123
23	4,898.534	6.52									
24	4,899.046	3.42									
25	4,900.377	12.85	4,900.393	H ₃ ⁺	4,900.138	4	6	+2	3	3	o 127
26	4,907.000	3.42									
27	4,907.859	7.90	4,907.869	H ₃ ⁺	4,907.381	1	3	+2	1	0	o 155
				H ₃ ⁺	4,907.827	3	0	-2	4	3	o 100
28	4,914.247	7.74	4,914.21 sh	H ₃ ⁺	4,913.634	3	3	+2	3	0	o 148
29	4,916.904	2.08	4,917.0069	H ₂				q-S ₁ (2)			
30	4,931.561	9.12	4,931.604	H ₃ ⁺	4,931.277	7	8	+2	6	5	p 118
31	4,935.946	8.12	4,935.969	H ₃ ⁺	4,935.667	3	5	+2	2	2	p 114
32	4,966.859	—	4,966.862	H ₃ ⁺	4,966.504	6	7	+2	5	4	p 111
33	4,971.537	—	4,971.559	H ₃ ⁺	4,970.645	2	0	-2	3	3	o 141
34	4,975.396	—									
35	4,997.561	—									
36	5,000.527	—	5,000.506	H ₃ ⁺	5,000.138	5	6	+2	4	3	o 101

List of the 36 new emission lines brighter than a 3σ threshold above the continuum, detected in the original spectrum of Jupiter before calibration, compared with the laboratory spectral lines and *ab initio* calculations. Missing intensities in Jupiter lines correspond to lines detected in the uncalibrated spectrum, but with large uncertainties in the calibration procedure (for weak lines or telluric line contamination). In the intensity fit two lines (4,820 and 4,931 cm⁻¹) are removed, because of probable interference by telluric and other jovian lines.

sh=shoulder on an H₂ line; o=ortho (nuclear spin weight 4); p=para (nuclear spin weight 2).

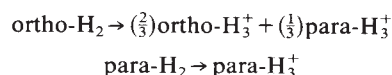
fundamental¹⁶. The high rotational temperature, however, together with the possibility that the lines might be due to neutral H₃, prevented definite assignments until improved *ab initio* predictions¹⁷ and an observation of the $2\nu_2 - \nu_2$ difference band¹⁸ became available. A model Hamiltonian¹⁹ fitted to these data allowed initial assignments. The *ab initio* calculations have now been extended to higher values of *J* (S.M. and J.T., manuscript in preparation), making it possible to assign a total of about 50 of the laboratory lines (A.M. *et al.*, manuscript in preparation) and 23 of the Jupiter lines, most of which coincide with a laboratory line. The Jupiter emission lines in this wavelength region therefore belong predominantly to the $2\nu_2$ band of H₃⁺ (Table 2).

The laboratory wavenumbers for the H₃⁺ lines were calibrated against lines of H₂ and have an uncertainty of the order of 0.01 cm⁻¹. A small residual line shift on the Jupiter lines can be accounted for by a slight offset (2 arcsec) of the aperture relative to the central meridian. The *ab initio* wavenumbers are on average 0.297 (±0.061) cm⁻¹ lower than the Jupiter wavenumbers, with a standard deviation of 0.297 cm⁻¹ about this mean.

Because the optical depth of the H₃⁺ lines is expected to be low (see below), the intensities of the lines are just proportional to the number of atoms in the upper states. The intensities were fitted following standard procedures of infrared spectroscopy²⁰,

allowing for a fraction f_p of para levels and $f_o = 1 - f_p$ of ortho levels. (The equilibrium values at high temperatures are $f_p = f_o = 0.5$.) Using the *ab initio* Einstein coefficients A_{ij} , the observed Jupiter intensities gave the rotational temperature, the para fraction f_p and the column density (that is, the concentration integrated along the path length). The best fit to the data of Table 2 gives: column density = $1.39 (\pm 0.13) \times 10^9$ cm⁻²; $T_r = 1099 (\pm 100)$ K; $f_p = 0.58 (\pm 0.03)$. The column density here refers to H₃⁺ ions in the $2\nu_2(l=2)$ vibrational state.

In the formation reaction for H₃⁺ (reaction (1) below), it is usually assumed that the weaker H₂⁺ bond is broken and that the ratios of the nuclear-spin modifications of the H₃⁺ produced therefore reflect those in the initial H₂ molecules according to the following relations²¹:



From the observed f_p of H₃⁺ we derive:

$$f_p(\text{H}_2) = [3f_p(\text{H}_3^+) - 1]/2 = 0.37(\pm 0.05)$$

This would be the equilibrium ratio at a temperature of ~106(±20) K, and is only a little higher than the measured

$f_p(\text{H}_2)$ in the troposphere²².

The primary mechanism for the production of H_3^+ in the atmosphere of Jupiter is the following reaction²³



(with $k = 2.0 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$, ref. 24). In the auroral region H_2^+ is produced principally by electron impact with H_2 (ionization by solar EUV is negligible):



The loss of H_3^+ occurs by the following dissociative recombination reactions:



or



The rate of reaction (3) is controversial at present, but the latest measurement is $1.8 (\pm 0.2) \times 10^{-7} \text{ cm}^3 \text{ s}^{-1}$ (ref. 25) which is in agreement with ref. 26 but not with ref. 27 ($\leq 10^{-11} \text{ cm}^3 \text{ s}^{-1}$). Using the larger rate constant the H_3^+ column abundance is calculated to be $6 \times 10^{10} \text{ cm}^{-2}$ in a non-auroral EUV-controlled ionosphere^{28,29}. In the jovian auroral region, however, $\sim 10^{13} \text{ W}$ of power is deposited²⁹, which corresponds to an energy flux of $10^{-6} \text{ W cm}^{-2}$. Ionization caused by the 1–10 keV electrons at this energy flux is expected to result in an H_3^+ column abundance as high as $6\text{--}10 \times 10^{12} \text{ cm}^{-2}$, with a factor of 2–3 uncertainty

resulting from uncertainties in the energy deposition rates, the chemical rate constants and the peak electron concentration. The maxwellian tail of the auroral electron energy distribution can provide the required ion-production rates above the ionospheric maximum. Assuming H_3^+ is the major ion, its column abundance is found to be $\geq 5 \times 10^{12} \text{ cm}^{-2}$ from the Voyager ionospheric measurements in the auroral region (67°S)³⁰. Using these estimates of the H_3^+ column density and the Einstein coefficients of Table 2, the optical depth in a strong H_3^+ line is found to be $< 10^{-3}$, as assumed in the intensity fit. The IUE emission reported in Table 1 is consistent with the model of ref. 29, from which the column abundance of H_3^+ is calculated.

There are two possible classes of mechanisms for populating the upper state of the transition. First, non-thermal processes: direct excitation mechanisms may be invoked, such as a transition between $\text{H}_2(v=1)$ (with a non-thermal population), and H_3^+ ; second, thermal processes—the population of the upper level could simply be the thermal population, in a high-temperature atmospheric level, with $T \approx 1,100 \text{ K}$. Thermal collisions are efficient only for large values of the parameter ϕ , the ratio of the inelastic-collision time to the radiative lifetime, derived from the Einstein coefficient. Using the rough estimates of $2.7 \times 10^{-10} \text{ cm}^3 \text{ s}^{-1}$ for the rate of vibrational quenching of H_3^+ by H_2 (ref. 31) and for atmospheric pressure between 1–100 nbars (ref. 29), ϕ is found in the range 0.1–4. Therefore, if the neutral temperature is as high as 1,100 K (ref. 29), a thermal population of the upper level of the $2\nu_2$ band of H_3^+ could account for the calculated column density of H_3^+ in the upper vibrational state. To discriminate between these possibilities further observations, such as the Doppler resolution of the H_3^+ lines (a measure of the translational temperature) or the observation of other vibrational bands of this ion, are required. □

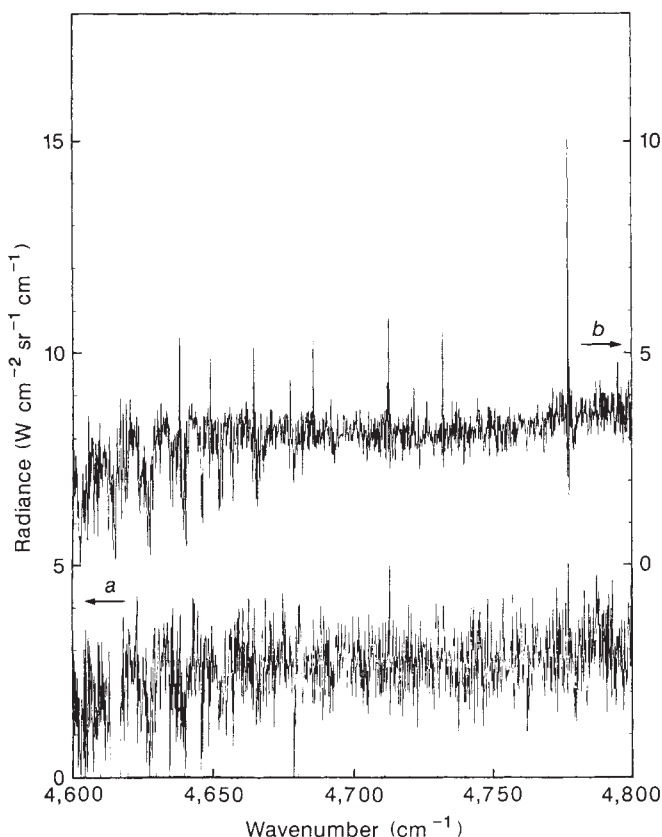


FIG. 1 FTS Spectra of Jupiter (24 September 1988, UT) recorded at constant latitude (60°S), with a 5-arcsec aperture kept centred on the central meridian of the jovian disk. *a*, For a non-auroral spot (longitude: 284° ; integration time: 13 min; signal to noise = 7) and *b*, for an average of the auroral spectra (mean longitude 60° ; total integration time: 90 min; signal to noise = 18). Spectral resolution is 0.23 cm^{-1} (FWHM). Calibration is performed by comparison with a standard star (θ_2 Tau, Bright Star 1412, K magnitude = 2.92).

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Iridium anomaly from the Acraman impact ejecta horizon: impacts can produce sedimentary iridium peaks

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THE observation of anomalously high iridium concentrations at the Cretaceous/Tertiary (K/T) boundary¹ triggered the still unresolved debate concerning the origin of sedimentary Ir anomalies. Several hypotheses have been presented, including meteorite impacts^{1,2} and intense volcanism^{3,4}. Here we present data from the Acraman impact ejecta horizon, South Australia⁵, which conclusively links high sedimentary iridium concentrations to a major meteoroid-impact structure and its widely dispersed ejecta. An Ir anomaly (up to 2 parts per 10⁹ (p.p.b.) Ir as well as Au, Pt, Pd, Ru and Cr anomalies) is present within the ejecta from the Acraman impact event preserved in late Precambrian (~600 Myr BP) shales within the Adelaide Geosyncline. The highest Ir (and Cr) concentrations within the ejecta are associated with the coarser-grained clasts of acid volcanics (boulder to medium-sand size) suggesting that most of the Ir is carried by these fragments. The target rocks at the impact site are comparable acid volcanics that have very low Ir, Au, Pt, Pd, Ru and Cr concentrations, indicating a meteoritic origin for the anomalous Ir levels within the ejecta. To our knowledge, this is the first example in which an accepted, widely dispersed impact-ejecta blanket, itself uniquely linked to a major terrestrial impact structure^{5,6} has been shown to be anomalously high in cosmogenic siderophile elements. This finding strongly supports the hypothesis that Ir anomalies in sediments can be produced by terrestrial meteorite impact events.

A problem with the meteorite impact scenario is that no suitably sized impact structure of end Cretaceous age has been found so far. Although a confirmed impact horizon has previously been found to contain high Ir⁷, no example of a terrestrial impact structure with widely dispersed Ir-enriched ejecta has been documented. The discovery of widely dispersed crustal ejecta linked to Australia's largest meteorite impact^{5,6} provides a unique opportunity to document the precious-metal signature of a demonstrable terrestrial impact-derived horizon.

Situated in the arid Gawler Ranges of South Australia, the Acraman impact site is underlain by a thick, acid volcanic sequence of mid Proterozoic (~1,600 Myr) Gawler Range volcanics. The final collapse crater may have been about 85 km in diameter, but the great age of the impact (~600 Myr) has resulted in several kilometres of erosion so that only a multi-ringed scar remains, centred on the Lake Acraman depression^{6,8}. The dimensions of the impact structure indicate a projectile some 4 km in diameter with a velocity of 25 km s⁻¹ and a density of 3 g ml⁻¹, which would have released energy equivalent to 5–10 × 10⁶ Mt (ref. 8) on impact. This major impact produced a widespread ejecta blanket which contains volcanic fragments up to 30 cm in diameter. The ejecta has been identified at distances >300 km from the impact site within the marine shales of the Bunyerroo Formation of the Adelaide Geosyncline⁵, and recently within the correlative shales of the Officer Basin in northern South Australia, about 450 km from Lake Acraman (M.W.W., V.A.G. and R.R.K., manuscript in preparation).

The Bunyerroo Formation containing the impact ejecta consists of monotonous greyish-red and greenish-grey shales, with minor concretionary carbonates. The ejecta horizon varies from

0 to 40 cm in thickness and, where hosted by greyish-red shales, almost invariably is enveloped by pale-green shales that range in thickness from a few millimetres to several metres. The ejecta horizon generally displays normal grading, with a basal monomict breccia of volcanic clasts overlain by progressively finer sands (Fig. 1). This is interpreted as a fall-out sequence which has been variably reworked by mass flow processes, storm waves and traction currents. All coarse fragments and most sand-sized grains, many of which show evidence of shock metamorphism⁵, seem to have been derived from a pink-to-red porphyritic volcanic rock similar to that currently exposed at the Gawler Ranges impact site. This correlation is supported by radiometric ages obtained from severely shocked but euhedral zircons within the ejecta⁹ (1,575 ± 11 Myr) which agree with the radiometric ages for the Gawler Range volcanics¹⁰ (1,592 ± 2 Myr). These ages contrast dramatically with the late Proterozoic (~600 Myr) stratigraphic age for the ejecta⁵.

We measured major and minor elements in the impact ejecta and its host shales from nine localities in the Adelaide Geosyncline and in seven samples of acid volcanics from the Acraman impact site using X-ray fluorescence analysis; the precious metals were determined by a combined NiS fire assay/radiochemical neutron-activation analysis technique¹¹. Precision was monitored by repeated analysis of an internal standard and the average coefficients of variation (standard deviation/mean) for Pd, Pt, Au, Ir and Ru were 5%, 10%, 20%, 7% and 21% respectively.

Figure 2 illustrates selected elemental profiles across the ejecta and host shales at Merna Mora Station (138°21.5' E, 31°33' S). Also illustrated are the average values for the host red shales. The ejecta horizon is anomalously enriched in Ir, Au, Pt, Pd, Cr and Ru. Iridium reaches a peak of 1.25 p.p.b. at the basal ejecta breccia, and the overlying ejecta sand has 0.46 p.p.b. Ir (Table 1). These ejecta values are 20–50 times greater than for the host red shales (average of 25 host red shales is 0.019 p.p.b. Ir). Ruthenium (not shown) is anomalously high in the ejecta (0.81 p.p.b. Ru) compared with the host shales (red-shale back-

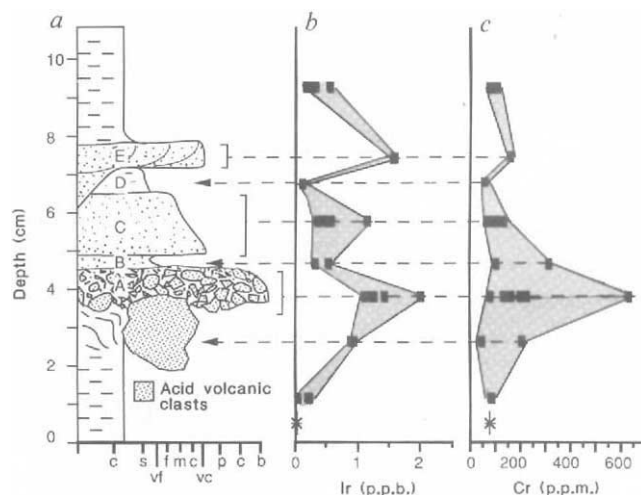


FIG. 1 Illustration of generalized ejecta stratigraphy together with the measured Ir and Cr concentrations in each layer (filled rectangles) from a number of localities. The most common sequence consists of, in ascending order, (1) breccia (A); (2) thin sandy mudstone (B); (3) graded sandstone (C). A fine silty horizon (D) occasionally overlies the graded sand unit. At one locality, a coarse cross-bedded sand overlies the graded sand unit (E). Shaded area indicates total range in Ir and Cr values. Ir and Cr data from Bunyerroo Gorge, Bunyerroo Gorge North, Brachina Gorge, Merna Mora Station, Brachina Gorge North, Parachilna Gorge, and Trebilcock Gap. Asterisks denote average values for host red shales (based on 25 red shale samples >1 m stratigraphically from the ejecta horizon). Abbreviations for grade scale are: clay; silt; very fine, fine, medium, coarse and very coarse sand; pebbles; cobbles; boulders.

TABLE 1 Analytical data for the Acraman ejecta horizon and host red shales of the Bunyeroo Formation and for the Gawler Range volcanics from the Lake Acraman impact site

Sample number	Locality	Ir p.p.b.	Au p.p.b.	Pt p.p.b.	Pd p.p.b.	Ru p.p.b.	Cr p.p.m.	Ni p.p.m.	Co p.p.m.	Zn p.p.m.
Basal ejecta breccias*										
808/93A	Merna Mora	1.25	0.31	3.46	1.00	0.81	631	52	11	14
8808/83A	Pichi Richi Pass	0.25	0.17	1.81	0.60	0.43	68	24	7	3
Ejecta sandstones*										
8808/93B	Merna Mora	0.46	0.11	1.48	0.37	0.07	89	31	7	17
8808/221	Wearing Hills	1.44	0.82	8.44	2.33	2.11	94	34	13	65
Yardea dacite from Acraman impact site										
8608/363	Southern Lake Acraman	<0.005	0.14	0.62	0.61	—	4	15	10	82
8608/365	Northern Lake Acraman	<0.005	0.25	1.14	0.57	—	3	9	7	95
8608/366	NW of Lake Acraman	<0.005	0.19	0.84	0.78	—	5	7	7	96
8808/267	Lake Acraman	0.01	0.15	0.68	0.13	0.03	—	—	—	—
Host red shale										
Average red shale (25 samples)		0.02	0.11	0.89	0.49	0.09	77	52	18	72
8808/155	Bunyeroo Gorge	0.03	0.08	0.53	0.44	0.07	82	61	20	121
8808/90	Merna Mora	0.004	0.05	0.56	0.34	0.05	76	49	13	15

* Ejecta samples with <100 p.p.m. Cu.

ground ~ 0.08 p.p.b.). Chromium is similarly enriched at the ejecta horizon, reaching a peak of 630 p.p.m. at the basal breccia. The target rock at the impact site (Yardea Dacite) has very low Ir, Au, Pt, Pd, Ru and Cr values (Table 1). The Yardea Dacite displays a relatively uniform character both vertically and laterally over distances >100 km and it is probable that the overlying (now eroded) dacites were similarly low in platinum group elements and Cr. Given that the host shales of the Bunyeroo Formation and the target rock at the impact site have very low Ir, Au, Pt, Pd, Ru and Cr abundances, the most likely source of these elements within the impact ejecta is the impactor itself.

When the siderophile element data for the ejecta horizon are Ir and C1-chondrite¹² normalized (Fig. 3), the majority of Ru, Au, Pt, Pd and Ni values fall in the range 0.6–6 times chondritic, indicating that the ejecta elemental ratios are similar but not identical to chondrites. The range of values for the Acraman ejecta samples overlap with those of the K/T boundary, although most Acraman values are slightly higher. These high values, as well as high Co and Zn normalized values, may be the result of high background values in the red shales hosting the Acraman ejecta combined with post-depositional redistribution of metals. Chromium values for the Acraman ejecta are, however, an order of magnitude higher than those of chondritic meteorites and for the K/T boundary. This could indicate that the chemical signature of the ejecta has been modified during diagenesis, a

possibility we are investigating (M.W.W., V.A.G. and R.R.K., manuscript in preparation).

Detailed examination of the Ir and Cr content of individual layers within the Acraman ejecta horizon at nine sites (Fig. 1) indicates that these elements are highest in layers with a high proportion of coarse-grained (boulder to medium-sand sized) material. The basal breccia almost invariably has the highest Ir concentrations (1–2 p.p.b. Ir) and analysis of individual breccia clasts demonstrates that these are also strongly enriched in Ir (1 p.p.b.). The sandy mud layer (volcanic clasts supported by shale) which commonly overlies the breccia is relatively low in Ir (0.3–0.5 p.p.b.) but the overlying sand layers tend to be higher (0.4–1.1 p.p.b. Ir). At one locality, the sand layer was overlain by a fine-silt layer which has a low Ir content (0.14 p.p.b.). Significantly, at one locality, the normally graded ejecta sequence is overlain by a layer of coarse cross-bedded and reworked ejecta sand (consisting of volcanic clasts) that is high in Ir (1.25 p.p.b.). The close correlation between coarse ejecta material and high Ir concentrations strongly suggests that most of the Ir is carried by the ejected clasts and/or clasts of devitrified glass. The shales directly overlying the sand and silt laminae generally have low Ir contents (generally ~ 0.2 – 0.3 and ranging up to 0.5 p.p.b.). The integrated Ir content of the ejecta generally range from 9 to 11 ng cm⁻² compared with an estimated average of 49 ng cm⁻² for the K/T boundary¹³.

The presence of an Ir anomaly within an accepted impact

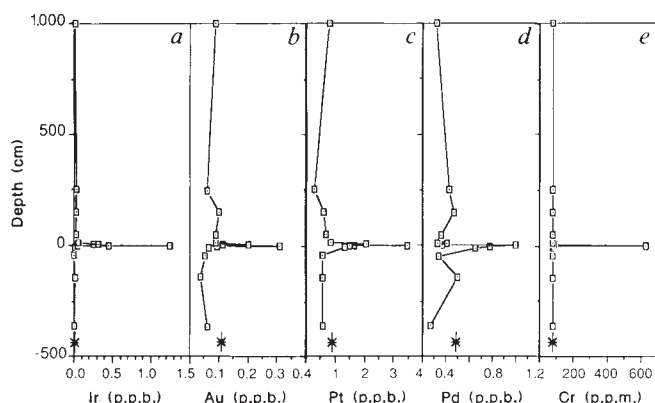


FIG. 2 a–e, Elemental profiles across the impact ejecta horizon at Merna Mora Station. Asterisks denote average values for host red shales (based on 25 red shale samples >1 m stratigraphically from the ejecta horizon).

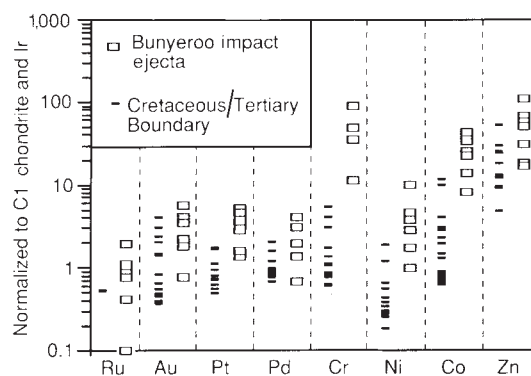


FIG. 3 Element/Ir ratios normalized to C1 chondrites for the Acraman ejecta horizon and for K/T Boundary samples. K/T boundary data taken from refs 2, 13, 14–18. Acraman ejecta samples from four localities within the Adelaide Geosyncline. Only samples with low (<100 p.p.m.) Cu contents were selected so as to minimize possible post-depositional contamination.

ejecta horizon that is linked to a large meteoroid impact structure gives strong support for the use of iridium as an indicator of major meteorite impact events in the geological record¹. In addition, we believe that the unique character of the Acraman ejecta horizon will permit correlation of late Proterozoic sediments not only from basin to basin, but between continents. Our recent discovery of the Acraman ejecta in the equivalent stratigraphic position in the Officer Basin has allowed us to correlate rocks in this basin with those in the Adelaide Geosyncline, up to 800 km apart (M.W.W., V.A.G. and R.R.K., manuscript in preparation). □

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Geotraverses across the Swiss Alps

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THE Alpine chain marks the collision zone between the European and African lithospheric plates. The Earth's crust below the Alps has been thickened by tectonic activity to form a 'root' of crustal material which extends to a depth of approximately 60 km. Here we present the initial results of three seismic reflection traverses across the Alps which show large-scale crustal indentations resulting from collision between continental plates, as well as the nappe-forming style of deformation produced by compression of attenuated ocean crust. The data suggest that the gross structural deformation of the collision zone results from large-scale crustal indentations. The descending European crust is delaminated and deformed by wedges of the African crust protruding northwards below the Alps. The nappe-forming deformation seems to be restricted to the upper 10–20 km of the Penninic domain in the core of the Alps.

Between 1986 and 1988, three major reflection traverses across the Alps were recorded (Fig. 1) for the NFP 20 research programme^{1–4}. They are linked together by two new refraction profiles measured using very close station spacing, parallel to the strike of the Alps. With CROP-Italia, additional wide-angle

shots were recorded across the mountain ranges along the Swiss/Italian border.

The classical cross-sections across the Swiss Alps constructed in the first half of this century have a depth range of 10–15 km (ref. 5). This was accomplished by cylindrical downward projection of surface structures along the plunging flanks of major axial culminations. During the past twelve years a new generation of lithospheric cross-sections has appeared^{6–12}, that are based on the results of seismic refraction and gravity measurements and modern methods of structural analysis, such as material balance. These extended the depth range to the base of the crust (50–60 km) and into the upper mantle.

In 1986 the first seismic reflection profiles were recorded across the Alps, namely the ECORS-CROP profile¹³ across the western Alps and the eastern traverse of NFP 20 across the Swiss Alps, the latter being an integral part of the European Geotraverse Project¹⁴. The standard field configuration consisted of a 240-channel spread laid out over 19.5 km. To ensure satisfactory results down to deep crustal levels, two independent data sets, one with vibrators and one with dynamite as seismic sources were acquired. Vibroseis profiling was carried out using a group of six 16-tonne vibrators. The data were recorded every 40 m to provide sufficient lateral and vertical data resolution for the upper 15 km of the crust. A very long 60 s sweep covering the frequency range 10–48 Hz resulted in optimum data quality. For deeper penetration, dynamite charges between 70 and 400 kg were fired at 5 km intervals to provide a continuous 2–4 fold coverage. This data set proved to be valuable for assessing the reflectivity and the gross features of crustal structure during the acquisition phase and for the initial stages of the data processing. The data processing has been carried out both at ETH in Zürich and the University of Lausanne. All of the sections presented here are unmigrated. Depth calculations are based on the assumption of an average velocity in the crust of 6 km s⁻¹, a realistic value that is based on seismic refraction measurements⁸.

In the northern Alpine foreland beneath the Swiss Molasse basin, the data exhibit a reflection pattern quite distinct from that found further north by DEKORP¹⁵ and to the west by ECORS^{16,17}. We observe (Fig. 2a,c) a clear band of strong reflections at the Moho level and a sporadically layered lower crust about 12 km thick with a rather transparent upper crust of somewhat greater thickness. The base of the sedimentary sequence at the southern margin of the Molasse basin appears at a depth of 7–8 km. Shallower reflections outline the Helvetic units that have been thrust over the central external massifs and now cover the southern part of the Molasse. The strong Moho reflections can be traced from a depth of approximately 35 km at the northern front of the Alps to about 55 km beneath the Penninic domain. High-energy explosion seismic data of this traverse⁴ show more continuous and better defined reflections from the lower crust and the Moho at the expense, however, of resolution in the upper shallower section.

The regular downward plunge of the Moho and the lower crust is one of the most conspicuous features on all of the profiles. As the European crust descends below the Alps (Fig. 2a,c) a remarkable thickening of the crust is observed beneath the external massifs. This structural anomaly appears to be caused by a northward protruding wedge of lower crustal material, which delaminates the plunging northern crust at a mid-crustal level. The upper European crust is detached from the subsiding lower crust, rolled back and bent upward^{6,8,19}.

There is good evidence for a similar indentation below the external massifs and the Penninic zone in the western traverse (Fig. 3a,c). This profile shows a conspicuous band of northward dipping reflectors below the base of the Penninic nappes and above the southward plunging lower European crust with the distinct band of Moho reflections. This reflection pattern also suggests that in this region the European crust is delaminated by a major wedge of lower crustal material, protruding northward below the external massifs and possibly reaching even

FIG. 1 Main tectonic units of a segment of the western Alps with the location of the three Swiss NFP-20 seismic transects (full lines) and the transects of CROP and ECORS (dashed lines). Seismic reflection data were recorded over a total length of 320 km. The west and south transects were done in four and five sections respectively.

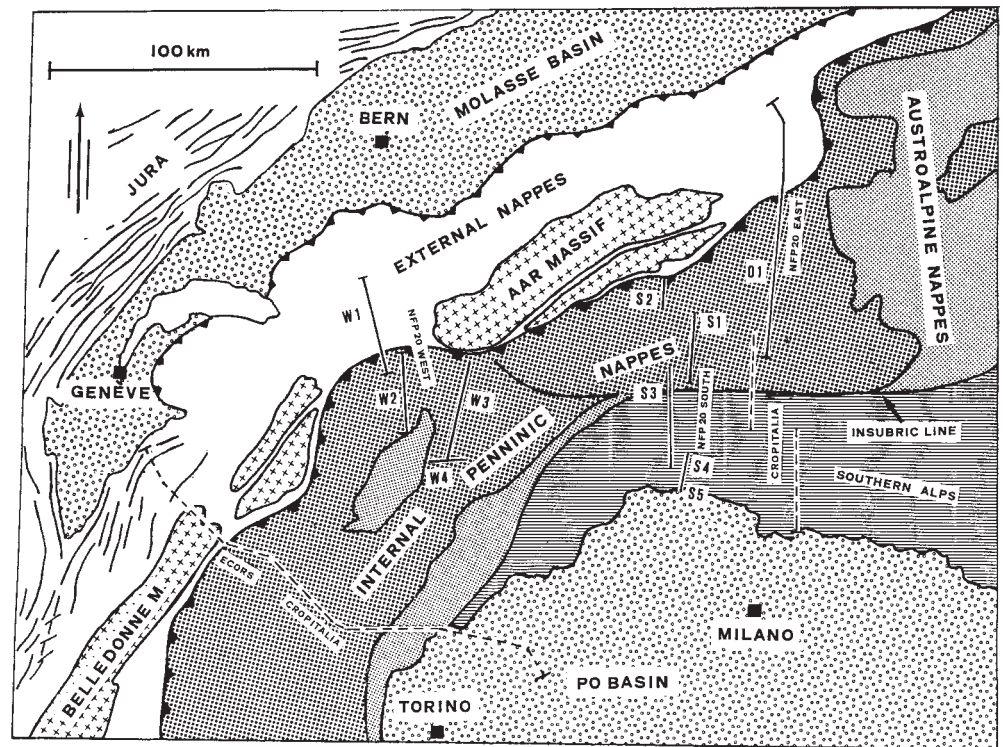
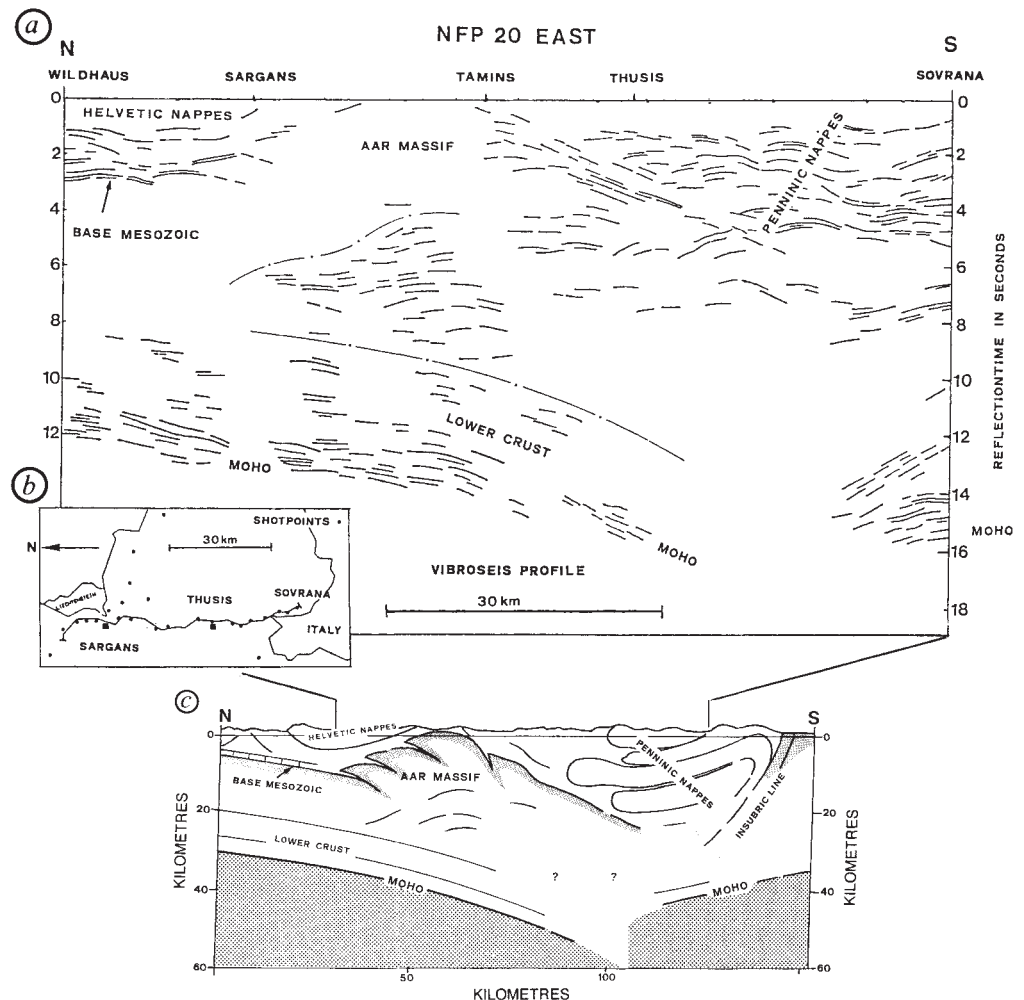


FIG. 2 *a*, Line drawing of the eastern Swiss traverse, NFP 20 East. A striking feature is the plunge of the European crust beneath the Alps. Below the massifs the crust appears split apart by an upthrust and northward protruding wedge of the lower crust. The Penninic front and the roof of the massifs are marked by strong reflections. The interbedded sequence of relatively flat gneissic nappes, sandwiched between Mesozoic sediments explains the excellent reflectivity of the Penninic domain. To obtain the approximate depth in km within the Alpine crust the reflection time must be multiplied by 3, because the average velocity, as indicated by seismic refraction, is $\sim 6 \text{ km s}^{-1}$. *b*, Location map of the eastern traverse. The filled circles are the shotpoints of the seismic explosions. *c*, The diagrammatic geological cross-section shows the position of the eastern Swiss traverse in the regional tectonic framework of the Alps.



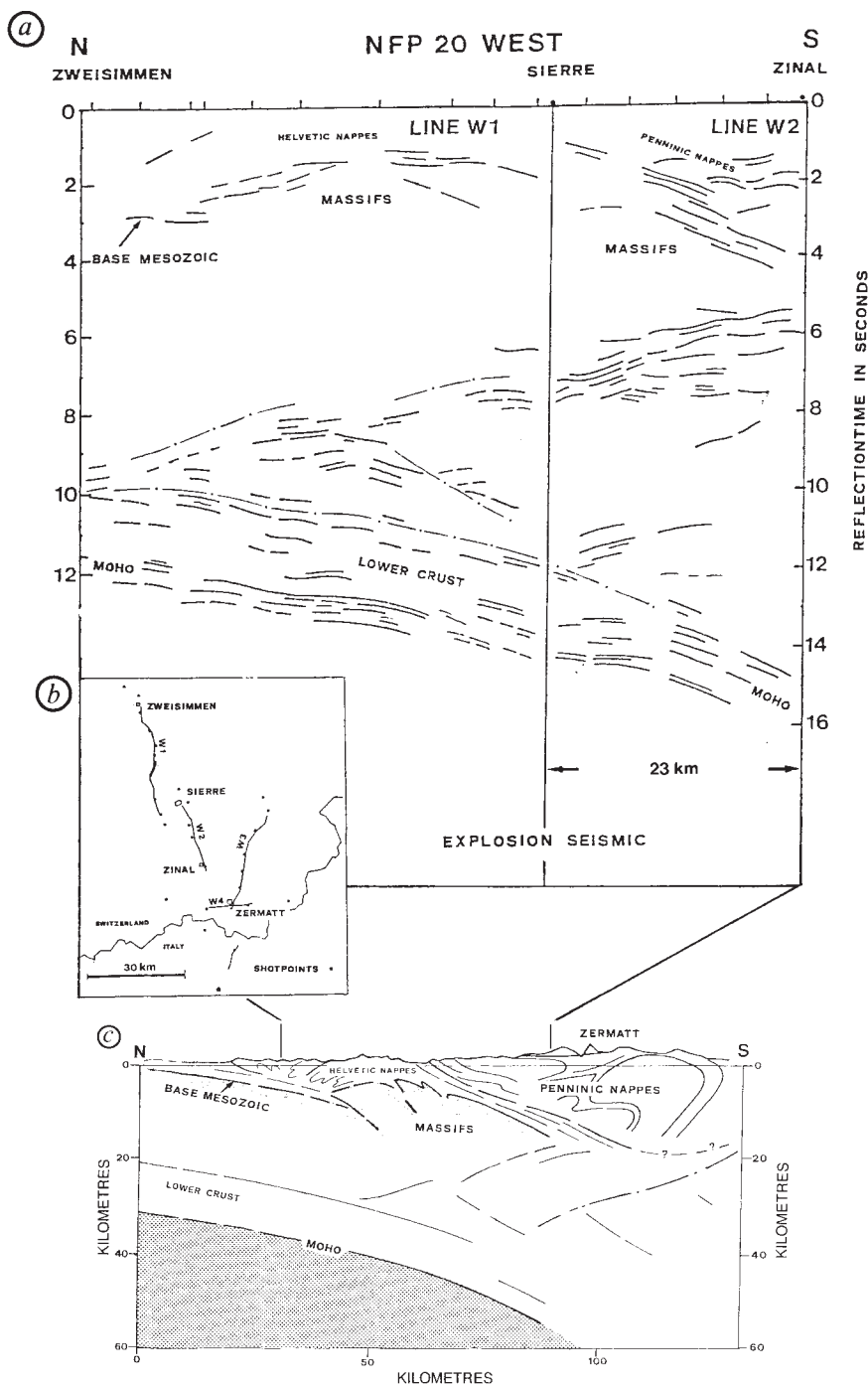


FIG. 3 a, Line drawing of the western Swiss traverse, NFP 20 West. This profile extends from the southern Molasse basin in the north to the Penninic zone near the Swiss-Italian border. In this region the massifs are crossed in an axial depression and carry the full load of the Helvetic nappes. Several prominent northward-dipping reflections can be recognized deep beneath the massifs and below the base of the Penninic nappes. They are interpreted as being related to the front of a major crustal indentation, which delaminates the descending European crust and forces the upper crust upward to form the massifs. b, Location map of the western traverse. The filled circles are the shotpoints of the seismic explosions. c, The diagrammatic geological cross-section shows the position of the western Swiss traverse in the regional tectonic framework of the Alps.

below the Helvetic domain. This wedge is probably related to the Ivrea body, a deep-crustal indenter below the western Alps, as predicted earlier¹⁸, from seismic refraction and gravimetric evidence. This tectonic style of crustal indentations has been dubbed the 'crocodile structure'^{19,20}.

Numerous strong and rather continuous reflectors appear in the Penninic domain to a depth of approximately 18 km (travel time 6 s) on the eastern and western traverses (Figs 2a,c and 3a,c). This domain is composed of gneissic nappes (flakes of crystalline basement), some 4–5 km thick, sandwiched between Mesozoic metasediments. There is a striking contrast between this thin-skinned, Penninic style of deformation and the large-scale crustal indentations below it. In this domain we may be observing the deformation pattern of the crust of the Tethys, stretched and thinned during Mesozoic time. The large-scale indentations are apparently the result of the collision of the two

continental plates below the Penninic zone and the external massifs.

At this stage only preliminary results of the explosion seismic data of the southern traverse are available (Fig. 4a,c). The transect crosses the Alps in a north-south direction somewhat to the east of the axial culmination between the eastern and western Alps. The structural units exposed at the surface in this region such as the Simano-Leventina gneisses, reach a depth of approximately 15 km, further east than the corresponding segment of the eastern traverse (compare Fig. 2a,c). The structural configuration of the southern traverse is dominated by a major crustal indentation. The lower crust of the southern Adriatic plate protrudes northward into the Penninic domain at a relatively shallow depth. Below the Penninic nappes the Moho of the descending European plate is seen at a depth of 55–60 km. The depth of the Moho at the southern end of the

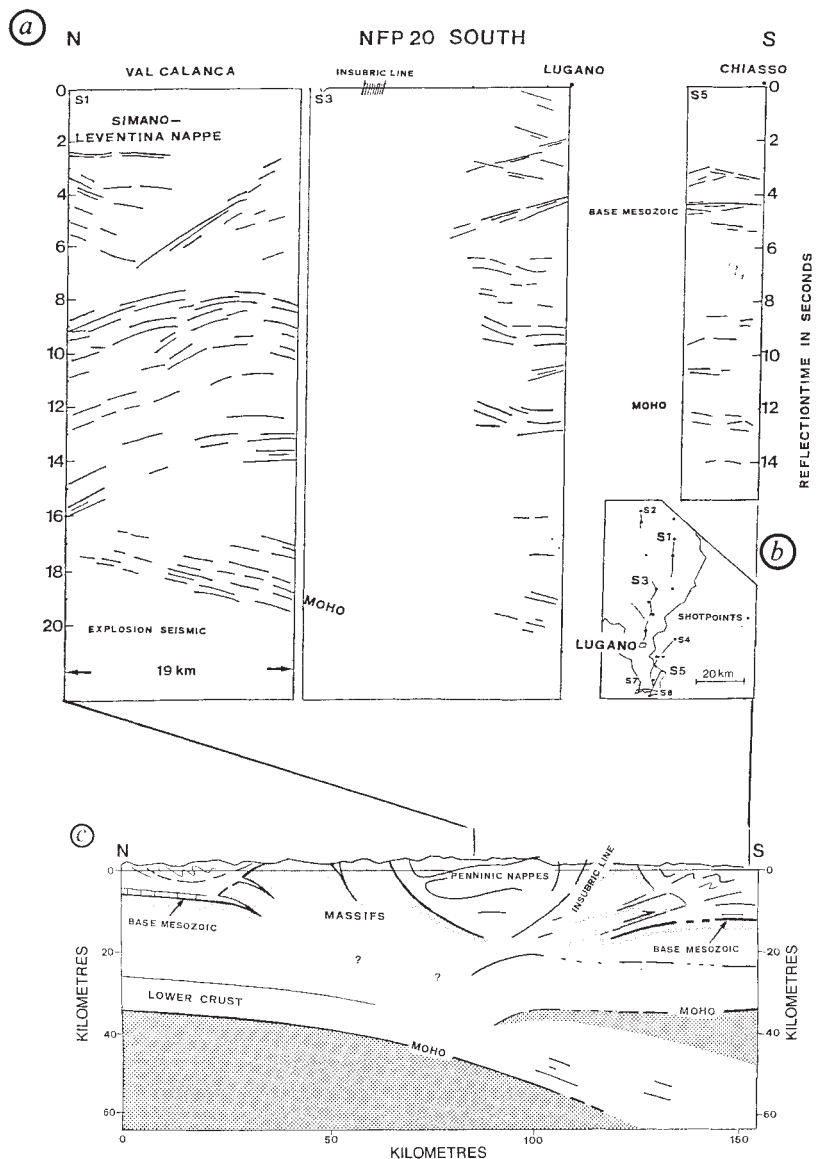


FIG. 4 *a*, Preliminary interpretation of the wide-angle explosion seismic data of a portion of the southern Swiss traverse NFP 20 South. The profile indicates that the lower portion of the Adriatic plate protrudes northward beneath the Penninic domain. This indentation could be responsible for the axial culmination of the Alps in this region. *b*, Location map of the southern traverse. The filled circles are the shotpoints of the seismic explosions. *c*, The diagrammatic geological cross-section shows the position of this explosion seismic profile in the tectonic framework of the Alps.

section and below the Penninic domain is confirmed by earlier seismic refraction work⁸. The upper crust of the southern Alps is highly structured. It appears to have been stripped off the lower crust below the Penninic domain and thrust southward. The basement of the southern Alps lies above a wedge of Mesozoic sediments, which plunges northward towards the Insubric line, as predicted earlier^{12,21,22}, based on material balance considerations.

Our data show the crustal indentations and delaminations that occur during continental collision. Large protruding wedges of 'exotic' crustal material apparently control the uplift of individual segments of the collision zones and seem to be responsible for the axial culminations.

A discussion of results and an exchange of data with our colleagues from CROP-Italia and ECORS is well under way. Their profile across the western arc of the Alps indicates a structure which is quite different from the one described in this paper. A striking feature on the Swiss profiles, for example, is the regular southward plunge of the lower crust and Moho of the European plate. By contrast, reflections on the ECORS/CROP-Italia profile from the Moho and the lower crust break up below the massifs and the crust/mantle boundary appears intensely imbricated¹⁶.

Seismic reflection data will be combined with other data (from studies of gravimetry, seismic refraction, isotope ages, aeromagneto-metry and satellite-based global positioning) in order to build an integrated model of the crustal structure of the Alpine collision belt. □

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Compositional constraints on the continental lithospheric mantle from trace elements in spinel peridotite xenoliths

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DATA for niobium in mid-ocean-ridge basalts (MORBs) and ocean-island basalts (OIBs) have provided new constraints on global geochemical models of continental growth and mantle differentiation. Until now, there were no such data in the concentration range of parts per 10^9 (p.p.b.) for peridotite samples. Here we report new measurements of Nb and other trace elements in fertile peridotite xenoliths. Our data show that the Nb/Ta ratio is chondritic, whereas Nb/Th (and presumably Nb/U) ratios are higher than chondritic. High Nb/Th ratios are characteristic of sources and/or residues of divergent-margin (for example, MORB) and intraplate (for example, OIB) magmatism. Compared with primitive mantle, these peridotites show a systematic and uniform depletion of the highly incompatible elements. The depleted patterns are apparently inconsistent with these peridotites being sources or residua from convergent-margin (that is, island-arc) magmatism. These data may indicate that the continental lithospheric mantle does not possess a Nb enrichment to complement the depletion found in the continental crust and island-arc basalts.

Peridotite xenoliths in alkali basalts can be used to obtain direct measurements of element abundances in the continental lithospheric mantle. The abundances of highly incompatible elements in these rocks are difficult to interpret because some of these elements were introduced by alteration processes (for example, K, Ba, Cs) and for other, more immobile elements (for example, Nb, Ta, Th) no reliable data exist because of the extremely low abundances of these elements.

We have selected five fertile spinel peridotites that were previously analysed for major and trace elements^{1,2}. Niobium and other trace elements were analysed by spark-source mass spectrometry (SSMS)^{3–5} and Ta by radiochemical neutron-activation analysis (RNAA)³. The results are presented in Table 1. This data set contains the lowest Nb and Ta abundances ever measured in geological samples. For some elements (for example, Ba and rare earth elements (REEs)), we have critically evaluated data from a number of techniques, wherever possible, to establish an internally consistent and high-quality data set.

The concentrations of the highly incompatible and relatively mobile elements (for example, Cs, Rb, K, Ba and U) in peridotite xenoliths may be affected by host infiltration and post-eruption alteration processes^{6–8}. It appears, however, that the relatively immobile elements (for example, REEs, Nb, Ta, Zr, Hf, Ti and Th) have not been significantly disturbed by alteration. Differences in the isotopic compositions of the xenoliths and their host basalts^{7,9} and the fact that most of these xenoliths have LREE (light REE)-depleted patterns, which strongly contrasts with the LREE-enriched character of the basalts, indicate that they have not experienced significant infiltration by their host magmas.

The ratios of Zr/Hf, Y/Ho and Nb/Ta are chondritic in basalts³ and komatiites¹⁰ with a wide range in concentration. These same ratios are also chondritic in the peridotite xenoliths (Zr/Hf = 35 ± 2 ($\pm 1\sigma$), Y/Ho = 26 ± 2 , Nb/Ta = 17 ± 3 and, in addition, Ti/Eu = $8,000 \pm 800$) but at much lower concentrations (Fig. 1). The constant ratios in both residues (depleted peridotites) and melts (komatiites and basalts) indicate that the elements in each pair have nearly identical solid/liquid partition coefficients during melting, in contrast to recent suggestions from experimental studies¹¹. Other careful analyses of peridotite xenoliths^{12,13} have also revealed chondritic Y/Ho ratios for incompatible-element-enriched and -depleted peridotite xenoliths. The reported deviations from non-chondritic Zr/Hf, Y/Ho and Nb/Ta ratios in peridotites may, in most cases, reflect errors in the analyses. For example, it has been recently demonstrated that X-ray fluorescence (XRF) calibrations for Nb and Zr analyses at low concentrations can easily yield erroneous results⁵.

In all of these relatively fertile peridotite xenoliths the Lu/Hf ratios (0.243–0.680) are greater than the bulk-earth (or chondritic) value (0.238). The variation in Lu/Hf ratios is about twice as large as the variation in Sm/Nd ratios (0.327–0.481). The magnitude and direction of this fractionation in both Lu/Hf and Sm/Nd and the relative fractionation between these ratios are consistent with the $\epsilon_{\text{Hf}}-\epsilon_{\text{Nd}}$ isotope array for oceanic basalts^{14,15} and would also be consistent with these peridotites being similar to MORB sources and/or OIB residues.

The constant non-chondritic Nb/U ratios in MORBs and OIBs have been interpreted as evidence for the homogenization of the mantle and subsequent formation of their mantle sources¹⁶. The continental crust¹⁷ shows a complementary depletion of Nb relative to the LREEs and U and this pattern is also found in island-arc basalts (IAB) and most continental basalts¹⁸.

TABLE 1 Trace element concentrations in spinel-bearing peridotites

	Sc-1	Po-1	Fr-1	D-1	KH-1	Method
Pb	0.12	0.19	0.11	0.088	0.11	1
Sn	0.14	0.14	0.21	0.093	0.11	1
Ba	2.62	4.33	1.89	3.4	2.42	1, 2, 4
K	137	33	9.6	36	6.5	1, 2
U	0.059	0.020	0.017	0.019	0.008	1
Th	0.032	<0.003	0.006	0.013	<0.003	1
Ta	0.0484	0.0020	0.0126	—	0.0013	2
Nb	0.698	0.036	0.193	0.353	0.029	1
La	0.48	0.106	0.326	0.213	0.063	1, 4
Ce	1.6	0.477	1.11	0.593	0.298	1, 4
Sr	29	8.73	15.6	6.29	5.52	1
Pr	0.268	0.103	0.176	0.085	0.070	1
Nd	1.35	0.619	0.911	0.409	0.488	1, 4
Zr	9.59	5.68	9.07	3.12	3.5	1
Hf	0.259	0.182	0.248	0.091	0.100	1, 3
Sm	0.441	0.298	0.341	0.142	0.233	1, 4
Eu	0.17	0.113	0.119	0.0503	0.087	1, 4
Ti	1,320	990	900	360	780	3
Gd	0.552	0.43	0.42	0.182	0.33	1, 4
Tb	0.099	0.081	0.075	0.036	0.068	1
Dy	0.688	0.546	0.502	0.245	0.482	1
Y	3.99	3.59	3.60	1.68	3.06	1
Ho	0.166	0.139	0.128	0.067	0.124	1
Er	0.485	0.427	0.409	0.215	0.384	1, 4
Yb	0.406	0.466	0.407	0.241	0.423	1, 4
Lu	0.063	0.069	0.066	0.037	0.068	1

All data are given in p.p.m. Methods used include: 1, SSMS; 2, RNAA; 3 and 4, instrumental neutron-activation analysis/thermal-ionization mass spectrometry. Accuracy and precision of SSMS and RNAA analyses in the p.p.b. concentration range are between 5 and 10%. Some of the data using methods 2 and 3 have been previously published^{1,2}. Locations for samples include: SC-1, San Carlos, Arizona, USA; KH-1, Kilbourne Hole, New Mexico, USA; Po-1, Potrillo, New Mexico, USA; Fr-1, Landoz, Massif Central, France; D-1, Dreiser Weiher, Eifel, FRG.

Because of alteration effects we cannot use the measured U abundances in the xenoliths to determine the mantle values of Nb/U. The Nb/Th ratios of these peridotites, however, can be compared with those of OIBs, MORBs, komatiites and IABs (Fig. 2)^{10,19}. The Nb/Th ratio in MORBs and OIBs is not as constant as the Nb/U ratio¹⁶ because of the slightly more incompatible behaviour of Th during melting and the differences in the Th/U ratios of the sources for MORBs and OIBs. Nb/Th ratios in these peridotites overlap those of MORBs and extend the OIB-MORB trend smoothly to higher values. They exceed the chondritic ratio by about a factor of three. The high Nb/Th ratios in some MORB samples probably reflect their source values and much of the upper mantle probably has a non-chondritic Nb/U ratio similar to those of MORBs and OIBs. The present upper mantle would therefore represent the residuum after the first differentiation of the upper mantle, which led to the formation of the continental crust.

To explain the similarity between these peridotites and the sources of oceanic basalts, we have calculated Nb/Th source ratios for MORBs and OIBs using the batch melting equation. The partition coefficients for Nb are assumed to be <0.01 in

clinopyroxene and garnet and the values for Th are 1–5 times less than those for Nb. These calculations demonstrate that although there is limited fractionation of Nb/Th between source and melt, severely depleted residues can have much higher Nb/Th values (Fig. 2). Our data suggest that peridotites with >100 p.p.b. Nb have Nb/Th values and Nb and Th concentrations comparable to those expected in the sources of MORBs and/or the residues of OIBs, whereas samples (Po-1 and KH-1) with low Nb concentrations of ~ 30 p.p.b. have compositions similar to those expected for MORB residues.

Figure 3 presents an overall view of the incompatible element abundances in the peridotites relative to primitive mantle. The most important observation is the systematic depletion of the most incompatible elements in the residual peridotites (KH-1, Po-1 and Fr-1). These depleted patterns can be produced by the loss of a partial melt enriched in incompatible elements, similar to that found in divergent-margin (such as MORBs and rift valley basalts) and/or intraplate (such as OIBs and continental intraplate basalts) lavas. Parallel incompatible-element patterns in some of these peridotites (such as Fr-1) and N-type MORBs (Fig. 3) suggest that these xenoliths are chemically analogous to MORB sources.

The trace-element composition of D-1 (not shown in Fig. 3) is similar to SC-1, but with lower absolute abundances. For a harzburgitic rock such as D-1 a flat incompatible-element pattern, particularly in the LREEs, reflects multistage evolution involving melt depletion followed by metasomatic-fluid enrichment¹. The relatively high Nb and Th concentrations for D-1 are also consistent with this model and suggest that the incompatible-element-enriched pattern of the metasomatic fluid was similar to that in alkalic basalts.

The chemistry of these peridotites reveals that they could be either the sources and/or residues from divergent-margin or intraplate magmatism. Salters and Shimizu²⁰, however, suggested that many peridotite xenoliths are depleted in high-field-strength elements (HFSE: Ti, Zr, Hf, Nb, Ta) relative to other incompatible elements and are from a world-wide, shallow, island-arc mantle source. Our whole-rock data do not support their suggestion that HFSE-depleted peridotites are widespread in the subcontinental lithosphere. Also, new results for D-1 (Table 1), a sample previously classified as a HFSE-depleted peridotite, show that the whole rock is not depleted in these elements, whereas the clinopyroxene has a HFSE-depleted pattern²¹. In contrast to the suggestion by Salters and Shimizu, we

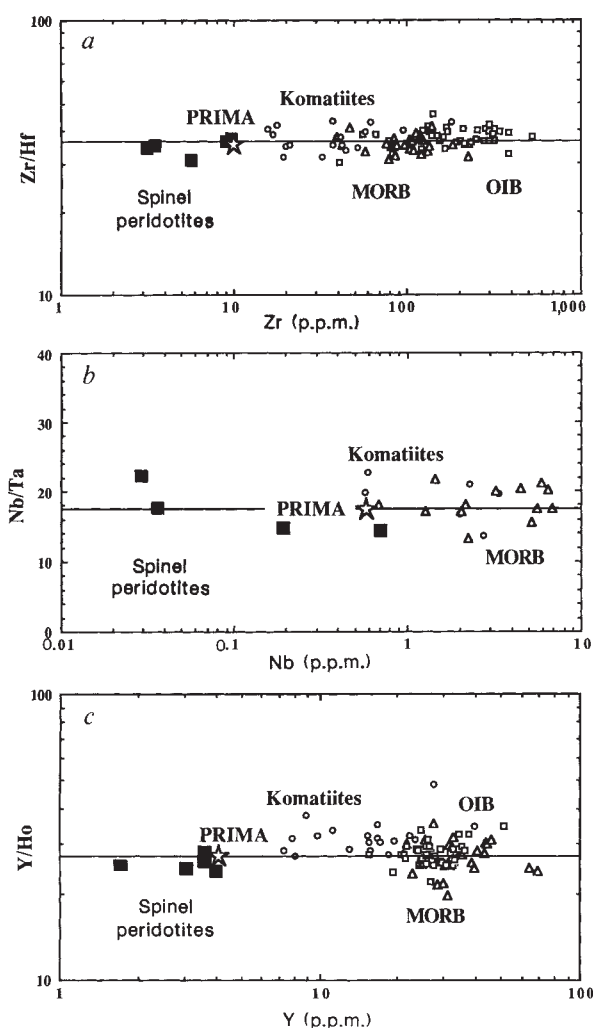


FIG. 1 *a*, Zr/Hf, *b*, Nb/Ta and *c*, Y/Ho ratios in peridotite xenoliths compared with MORBs, OIBs and komatiitic lavas^{3,10,16,27}. Primitive mantle (chondritic) values are indicated by the open star and are labelled PRIMA (36, 17 and 28, respectively). These data demonstrate that the peridotites, which can be the potential sources and/or residues for a wide variety of lavas, possess chondritic values of these ratios that are the same as in the basalts and komatiites. This argues for similar partitioning of the elements in each pair during melting and over geologic time.

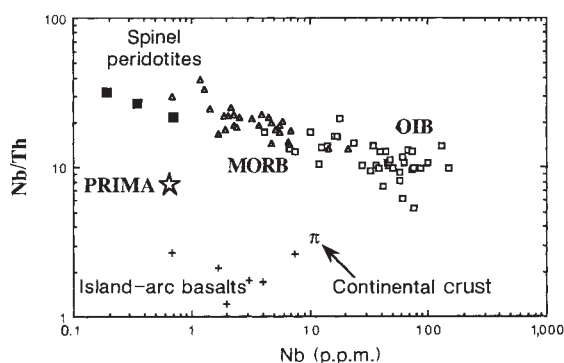


FIG. 2 A plot of Nb/Th against Nb concentrations in peridotite xenoliths, MORBs, OIBs and komatiitic lavas^{3,10,16,27}. The overlap in Nb/Th ratios for peridotites and the MORB-OIB array contrasts with that seen in the continental crust and island-arc basalts. Nb/Th ratios in the sources of MORBs and OIBs and in the lavas are comparable with those in peridotites with more than 100 p.p.b. Nb. Residua of MORBs and OIBs as well as the more residual peridotites reported here are suggested, however, to have higher Nb/Th values and very low Nb contents compared with an initial source composition. Data sources are as in Fig. 1, except for the continental crust³ and island-arc basalts (unpublished data, K.P.J. *et al.*).

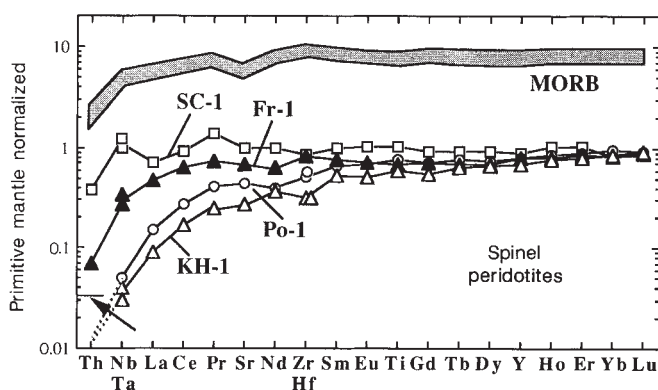


FIG. 3 Primitive, mantle normalized abundance patterns for the incompatible elements in peridotite xenoliths. The abundance pattern for an average N-type MORB²⁸ is similar to that of Fr-1, but about a factor of ten higher in concentration. Primitive mantle normalizing values are from ref. 29. The order of incompatibility of the elements is that which is used for MORBs. Arrow, detection limit for Th.

conclude that the incompatible-element pattern of clinopyroxenes does not necessarily represent that of the whole rock. Island-arc basalts are depleted in some HFSEs (possibly not Zr and Hf) relative to the REEs according to the element sequence in Fig. 3. Given a HFSE-depleted characteristic of an IAB, we would then expect either comparable HFSE depletions in these peridotites or enrichments in these elements relative to the REEs, as might be predicted for an IAB residue. This is not the case here. In addition, relatively primitive IABs have high, non-chondritic Sr/Nd ratios (30–35), whereas primitive MORBs (10–15) and OIBs (14–22) have near chondritic (15.5) values. The xenoliths studied here have an average Sr/Nd of 16 ± 4 , which is identical to the global average of 15 ± 5 for Sr/Nd (ref. 22) in peridotites. In combination, these data therefore argue against these peridotites being either sources or residua of island-arc magmatism.

It has been proposed that the continental lithospheric mantle grows by the underplating of refractory peridotite diapirs^{23–25} produced during magmatism; that is, the extraction of basaltic melts from ascending diapirs produces residual less-dense peridotite bodies that are buoyant with respect to the surrounding mantle and therefore gravitationally stabilized beneath continents. This raises the question of the type of tectonic environment in which most growth of the continental lithospheric mantle occurred. Our results indicate that these peridotites lost a basaltic melt that could have been produced in a divergent-margin and/or intraplate tectonic setting and are not consistent with them having lost an island-arc type (that is, a convergent-margin) melt.

Finally, these peridotites have been used to construct primitive-mantle Earth models^{1,2}. The high and non-chondritic Nb/Th (and probably the unaltered Nb/U) ratios in these peridotites are similar to oceanic basalts and not to the primitive mantle. Depletions in all of the most incompatible trace elements (Fig. 3), non-chondritic Ca/Al (ref. 26) ratios and non-bulk-Earth Sr and Nd isotopic compositions⁹ together demonstrate that these samples have experienced complex histories. Thus, as concluded previously^{1,2,9}, such samples should not be used to derive primitive-mantle compositions for highly incompatible trace elements. □

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Age of Allan Hills 82102, a meteorite found inside the ice

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THE recovery of thousands of meteorites in the Antarctic since 1969 has not only greatly increased knowledge of the meteorites themselves but has also provided a new tool for glaciology. Most Antarctic meteorites are found on blue ice areas where old ice is continuously ablated. A measurement of the age of this ice helps us understand meteorite accumulation mechanisms and the dynamics of ice movement. The terrestrial age of a meteorite, the time period since the date of meteorite fall, can be determined from the reduction in concentration of cosmogenic radionuclides during the time the meteorite has been shielded by the Earth's atmosphere. Here we report the terrestrial age of a meteorite that was recovered from below the surface of the ice and argue that this represents a measurement of the age of the ice itself. We measured the cosmogenic radionuclides ¹⁰Be (half-life = 1.5 Myr), ¹⁴C (5,730 yr), ²⁶Al (0.71 Myr), ³⁶Cl (0.30 Myr) and ⁵³Mn (3.7 Myr) in the meteorite and ¹⁰Be and ³⁶Cl in the ice. We obtained a terrestrial age of 11,000 years for the meteorite which suggests that the snow accumulation area where it fell was only a few tens of kilometres away.

Existing methods of dating old ice are radiocarbon dating of trapped CO₂ and uranium-series dating of dust embedded in the ice¹. Radiocarbon dating is possible to only about 25,000 yr, presently requires about 5 kg of ice, and involves a significant probability of contamination for ice near the surface. Although the uranium-series dating is not yet well tested, the method has been applied to several Antarctic ice samples¹. Current under-

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standing of the meteorite accumulation mechanism (see for example, refs 2-4) provides the following scenario. The meteorites fall into a snow accumulation zone and become buried in the snow, which under growing pressure becomes firm and then ice. They are then transported by iceflow to the edge of the Antarctic continent. Where the ice movement is blocked by obstacles such as the Allan Hills and the Yamato mountains, the flow stagnates. In such places the ice is continuously ablated by wind and the meteorites 'weather out' and remain exposed on the surface. The terrestrial ages of the meteorites found on the ice do provide some limiting information on its age. The best samples for studying this problem are those few meteorites actually found embedded in the ice. In these cases the terrestrial age of the meteorite should be the same as the age of the ice, unless recent remelting allowed it to sink back into the ice.

Only 4 out of over 10,000 Antarctic meteorites have been found below the surface of the ice: one at Allan Hills, two at Yamato, and one at Lewis Cliff. Allan Hills 82102 (ALH82102) was the first meteorite to be collected with the surrounding ice intact. The meteorite was found in the Allan Hills Far Western icefield at 157°14'30" E, 77°2'33" S, about 70 km west of the Allan Hills themselves and of the Allan Hills Main icefield, where over 2,000 meteorites have been found during the past 10 years. The locations of the meteorite and the Allan Hills icefields are shown in Fig. 1. The meteorite was collected *in situ* in a large block of encasing ice (field No. 2995) by the 1982-83 US expedition team. The specimen inside the original ice block was sent to the Snow and Ice Branch, Cold Regions and Engineering Lab, Hanover, New Hampshire. Extreme care was taken to preserve the orientation of both ice and meteorite. The crystalline structure of ice in immediate contact with the meteorite was examined in orthogonal thin sections and no structural characteristics to indicate melting and refreezing or annealing were found⁵. It was concluded that the meteorite was just beginning to emerge at the ablation surface and that the ice enclosing the meteorite was coeval with the terrestrial age of the meteorite⁵. One half of the ice was sent to La Jolla for this study.

ALH82102 is an H5 chondrite with a recovered mass of 48.0 g (3×4×4 cm). Our meteorite sample was ground and separated into magnetic (19%) and non-magnetic (81%) fractions. The metallic phase was obtained by further purification of the magnetic fraction. The nuclides measured were ¹⁰Be, ²⁶Al, ³⁶Cl and ⁵³Mn in 148 mg of the metallic phase (Fe 89.9%, Co 0.51%, Ni 9.32%), ¹⁰Be and ²⁶Al in 120 mg of the bulk sample (Al 0.98%, Mn 2210 p.p.m., Fe 32.1%, Ni 1.86%), and ¹⁴C in the silicate phase. The ¹⁰Be and ²⁶Al measurements were carried out by accelerator mass spectrometry (AMS) using the tandem van de Graaff accelerator at ETH, Zürich⁶. The ¹⁴C measurement was carried out by AMS at the University of Arizona⁷. ³⁶Cl was determined by AMS using the University of Rochester MP tandem van de Graaff accelerator⁸. ⁵³Mn was measured by

neutron activation using the National Institute of Standards and Technology (former NBS) reactor at Maryland. The results are shown in Table 1. Small corrections have been made to the ¹⁰Be and ²⁶Al concentrations in the metallic fraction because of the small amount of silicate in it. The ²¹Ne exposure age of the meteorite is 7.1 Myr (L. Schultz, personal communication).

The terrestrial age of the meteorite can be calculated from the ³⁶Cl and ¹⁴C activities. The nearly saturated ³⁶Cl activity indicates a terrestrial age of 50,000±50,000 yr. The ¹⁴C released in the >500 °C extraction fraction is 13.9±0.3 d.p.m. per kg silicate (d.p.m.=disintegrations per minute). The saturation activity measured in the same temperature fraction for bulk H-chondrites is 43 d.p.m. kg⁻¹ (±16%) (ref. 7). Most ¹⁴C is produced in the silicate phase of chondrites because the major target element for its production is oxygen. The typical ¹⁴C concentration in iron meteorites is ~2 d.p.m. per kg metal. As the silicate fraction used for the ¹⁴C measurement was 81% of the mass of the ALH82102 sample, the 43 d.p.m. kg⁻¹ saturation activity for bulk H-chondrites should be increased to 53 d.p.m. kg⁻¹ for comparison with the activity measured for the silicate phase of this meteorite. From this, we calculated a ¹⁴C terrestrial age of 11,000±1,300 yr.

As the meteorite was embedded in the ice, the surface of the ice in the Allan Hills Far Western icefield can be estimated to be ~11,000 yr. We believe this is the first direct age determination of ice by the terrestrial age of a meteorite. Even though the survey of ice movement for the Allan Hills Far Western icefield has not yet been completed, the age of ice obtained here suggests that the major accumulation area for the ice is a few tens of kilometres south-west of the Far Western icefield, according to the ice dynamics model⁴. The relatively close and limited extent of the ice catchment area of ice flowing towards this area has also been suggested by Drewry⁹. Most of the meteorites found in the Allan Hills Far Western icefield have terrestrial ages <60,000 yr¹⁰. The terrestrial age of ALH82118 which was found ~650 m east of ALH82102 is 21,000 yr¹¹. The terrestrial age of ALH83102 which was found ~2 km west of ALH82102 is 10,000 yr¹². The age of the Allan Hills Main icefield, where many meteorites with terrestrial ages >200,000 yr have been found, is estimated to be 100,000-400,000 yr based on uranium series dating¹ and the general trend of the terrestrial age of meteorites¹⁰. The 'young' age of the Allan Hills Far Western icefield compared to the 'old' age of the Allan Hills Main icefield is also supported by the difference of meteorite concentrations in both icefields, although precise counts are not available.

The ice sample No. 2995 was sectioned horizontally to give samples from different depths: 0-10 cm (2,481 g) and 10-17 cm (2,525 g) below the surface. For comparison a one-day-old (5 January, 1984) snow sample (3,841 g) was collected from the Allan Hills Middle Western icefield (158°10'0" E, 76°49'0" S, see Fig. 1) by one of the authors (K.N.). ¹⁰Be and ³⁶Cl in the two samples of ice No. 2995 were (9.26±0.41)×10⁴ atom ¹⁰Be g⁻¹ and (5.13±0.36)×10³ atom ³⁶Cl g⁻¹ for the 0-10 cm sample and (13.50±0.47)×10⁴ atom ¹⁰Be g⁻¹ and (4.97±0.46)×10³ atom ³⁶Cl g⁻¹ for the 10-17 cm sample. The snow contains (1.43±0.09)×10⁴ atom ¹⁰Be g⁻¹ and (4.32±0.35)×10³ atom ³⁶Cl g⁻¹. The ¹⁰Be and ³⁶Cl in the ice and snow were produced by cosmic-ray interactions with atmospheric nitrogen and oxygen

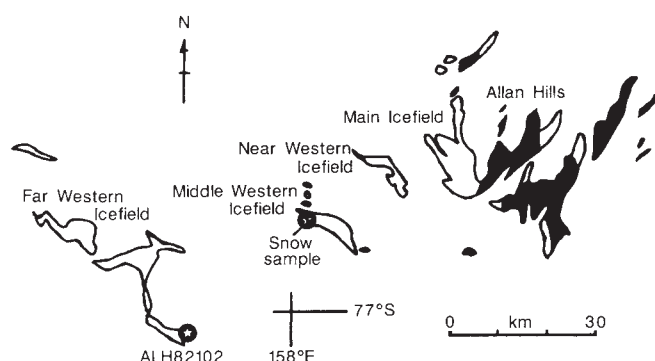


FIG. 1. Map of the Allan Hills icefields, Southern David Glacier region, Victoria Land. The black portions indicate exposed bedrock. The sample locations of ALH82102 and snow sample are indicated with asterisks.

TABLE 1 Cosmogenic nuclide concentrations in ALH82102

	¹⁰ Be	¹⁴ C	²⁶ Al	³⁶ Cl	⁵³ Mn
Phase	(d.p.m. kg ⁻¹)	(d.p.m. kg ⁻¹)	(d.p.m. kg ⁻¹)	(d.p.m. kg ⁻¹)	(d.p.m. per kg (Fe + 1/3 Ni))
Metal	5.31 ± 0.15	—	3.80 ± 0.28	21.65 ± 0.87	249 ± 9
Bulk	14.49 ± 0.38	13.9 ± 0.3*	30.9 ± 2.1	—	—
			39 ± 2†		

* Silicate phase.

† Ref. 15.

for ^{10}Be and argon for ^{36}Cl . After production both nuclides become attached to aerosols and are removed by precipitation. The ^{10}Be concentrations in samples of the same ice block No. 2995 differ by $\sim 60\%$. The average accumulation rate for the Allan Hills area is $\sim 4\text{ cm (ice equivalent) yr}^{-1}$ (ref. 3). If the age of the ice is a simple function of depth, each ice sample contains 2–3 years accumulation of snow. Precipitation may not accumulate continuously, however, due to the strong wind. The difference of ^{10}Be concentrations in the top and bottom of the ice block can be explained by the variation of ^{10}Be production rate during the solar cycle and terrestrial influences such as a change in precipitation rate. However, the higher ^{10}Be concentrations in the ice compared to fresh snow in the same area (about a factor of 6–9) cannot be explained by the solar cycle.

Our measurement of the age of surface ice in the Allan Hills Far Western icefield, 11,000 yr, is the time of the end of the Glacial/Holocene transition. An increase of ^{10}Be concentration by more than a factor of two between about 11,000 yr and 14,000 yr is well known in both Antarctic and Greenland ice cores (for example refs 13,14). Raisbeck found $\sim 10\text{--}12 \times 10^4$ atoms $^{10}\text{Be g}^{-1}$ in a Dome C ice core from the last ice age¹³.

Although Dome C is located $\sim 1,000\text{ km}$ inland from the Allan Hills, the data for Dome C could apply because the climate change was world wide. $\square$

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Assessment of effects of socio-economic status on IQ in a full cross-fostering study

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AN important question in studies of mental ability concerns the effect of parental socio-economic status (SES) on the IQ of their offspring. Only a full cross-fostering study, including children born to biological parents from the most highly contrasting SES and adopted by parents with equally contrasting SES, can answer this question. Previous adoption studies using incomplete cross-fostering designs^{1–3} have indicated an effect of postnatal environment on the IQ of children born to low-SES backgrounds and adopted by high-SES parents. They have not shown whether a low SES reduces the IQ of children born to high-SES parents or whether the SES of biological parents has an effect on IQ, or whether the effect of the SES of adoptive parents is independent of the SES of biological parents. We present a full cross-fostering study dealing with IQ, and find that children adopted by high-SES parents score higher than children adopted by low-SES parents; children born to high-SES parents score higher than children born to low-SES parents; and that there is no evidence for an interaction between these two factors on children's IQ.

Data reported elsewhere in the literature^{1,2,4} have been arranged *a posteriori* in the form of full designs (refs 5 and 6; and S. Scarr, personal communication). The results of two of these designs^{5,6} where the two factors (biological and adoptive parents) were defined by the same variable—number of years schooling (NYS)—are contradictory. One study showed an effect on children's IQ only for NYS of biological parents⁵, whereas the other reported an effect for the NYS of adoptive parents⁶. This contradiction probably results from the arbitrary definition of levels of the independent variables. In addition, the contrast in levels between the characteristics of the groups of biological parents, as for the adoptive parents in the samples, tends to be low. This is why an *a priori* cross-fostering study was designed.

The sample was composed of four groups of adopted children (Table 1). The two-by-two factorial design includes two independent variables (SES of the two biological parents and of the two adoptive parents) each split into two highly contrasted levels (highest versus lowest). The two factors were defined operationally in terms of SES, by a combination of NYS and occupational status. Subjects were selected from two private adoption agencies and six public agencies according to the stringent procedure⁷ developed for French adoption studies^{3,8}. Subjects were born between 1 January 1970 and 31 December 1974. The range of birth dates was later extended to 31 December 1980 to obtain B+/A– subjects as these are extremely rare (1 of 600 files). The extension of the birth-date range for this latter group does not introduce a bias because all the subjects meeting the requirements in the total population of each agency were included in the study. Before beginning the screening, we decided that 10 subjects per group was statistically sufficient. The strict selection procedure will be described as it justifies the use of small sample sizes. Of 380 children relinquished at birth in the first adoption agency, 10 files met the requirements for group B–/A–, 10 for group B–/A+, 8 for group B+/A+ and 3 for group B+/A–. The search for B–/A– and B–/A+ subjects was, therefore, stopped. In Agency 2, out of the 146 children relinquished within the time period set by the study, 1 B+/A– and 10 B+/A+ subjects met the criteria. Only 2 B+/A+ were selected after matching with characteristics of A+ parents of the B–/A+ group. Four B+/A– were selected from six other public agencies. The likelihood of introducing non-systematically controlled variables having an impact on IQ is extremely low with this procedure. Furthermore, the two-way analysis of variance allows for calculating factor effects by augmenting the number of degrees of freedom.

The occupations of each of the two biological and adoptive low-SES parents corresponded to the lowest level on the scale, and the occupations for each of the two biological and adoptive upper-SES parents corresponded to the highest level (Table 1). Graduates (at least 13 years of schooling) were included in the group of highest biological background parents. NYS means are identical for the two groups of B– parents and the two groups of A– parents, and identical for the two groups of B+ parents and the two groups of A+ parents, whereas the differences were considerable between the + and – groups. Moreover, within-group variance of NYS was extremely low for all groups (Table 1). Control for possible bias showed no evidence of

selective placement: the age of the child at the time of relinquishment and age at adoption did not differ between the four groups; and as the within-group variance for SES and NYS was very low, no selective placement could have taken place on the basis of these criteria in this study. No significant differences were observed for the four groups for length of gestation, birthweight, or the prevalence of neo- or perinatal disorders. All the subjects selected from their files were subsequently tested. Therefore there was no bias due to loss of subjects. The mean age of subjects at the times of testing (168.10 ± 3.28 months) did not differ among groups.

Testing and scoring for calculation of the Full IQ were carried out using a blind procedure, as the testers did not know that the study dealt with adopted children. Each adoptee and one classmate were individually administered the Wechsler Intelligence Scale for Children-Revised (WISC-R⁹) in his or her regular school. Each test was scored twice; first by the tester, and secondly by another psychologist uninformed of the aims of the study. Only the second scoring was included in data analysis.

Full IQ scores (Table 2) show an effect for SES of the adoptive parents on their adopted children's IQ. Children reared by high-SES parents have significantly higher IQs than those reared by low-SES parents. Thus, there is a postnatal environmental effect on IQ performance which is independent of the SES of the biological parents. Partial comparison (Table 2), which reproduces the change of background comparable to the first partial French adoption study³, show the same directional effect as those in this study³. The previous study indicated that mean full IQ WISC¹² scores for children born to low-SES parents and adopted by high-SES parents are significantly higher than those obtained by their non adopted half-siblings who remained in their original backgrounds. This partial design, however, is

TABLE 2 IQ of adopted children

	SES of adoptive parents		
	High A+	Low A-	
SES of biological parents	n=10	n=8	113.55
	$\bar{x}$, 119.60	$\bar{x}$, 107.50	
	σ , 12.25	σ , 11.94	
	Range, 99-136	Range, 91-124	
	n=10	n=10	98.00
	$\bar{x}$, 103.60	$\bar{x}$, 92.40	
	σ , 12.71	σ , 15.41	
	Range, 91-125	Range, 68-116	
111.60		99.95	

An analysis of variance (unweighted means) on full IQ scores indicates significant effects of both biological and adoptive parents' SES. Effect of adoptive parents' SES, ($F(1, 34) = 7.31$; $P = 0.010$), mean difference 11.6 IQ points in favour of children adopted by high SES parents. Effect of biological parents' SES, ($F(1, 34) = 13.02$; $P < 0.001$), mean difference 15.5 IQ points in favour of children born to high SES parents. The interaction for these two factors is not significant ($F(1, 34) = 0.011$). Nevertheless, a partial analysis of B-/A- and B-/A+ subjects is warranted since a directional hypothesis based on results of a previous study³ can be formulated. The results show a partial effect for the adoptive parents' SES on the IQ of B- children: $t(18) = 1.773$; $P < 0.05$, one tailed-*t*-test.

uninformative as to whether the improvement in observed scores was due to postnatal environment as assessed by the parental SES, or to the adoptive parents providing a more stimulating child-rearing environment. By testing adopted children only, the present study provides new and original data showing that improvement in performance is clearly caused by change—low SES versus high SES—in the postnatal environment.

The data show an effect for the biological parents' background on IQ independently of the SES of the adoptive parents (Table 2); that is, children born to parents with high SES have significantly higher scores than children born to low-SES parents. Although these findings clearly indicate that the biological parents' background contributes to observed differences in IQ between extreme groups, as does that of the adoptive parents, more detailed interpretation is difficult. The adoption method provides a means of dissociating the pooled effects of genetic and prenatal factors from factors related to the postnatal environment. But it is not equipped to differentiate prenatal from genetic factors¹⁰. This precludes interpreting the effects of the biological parents' background solely in genetic terms, or concluding that observed effects could be prenatal or prenatal acting either in additive or interactive manner with genotype. On the contrary, the effect attributed to the adoptive parents is clearly environmental.

The absence of an interaction effect calls for comment. The groups selected for this study represent extremes, that is, those with the greatest likelihood of exhibiting an interaction, if indeed one is present. The fact that there is no interaction is convincing evidence that changes in IQ resulting from changes in postnatal environment are of similar magnitude and exhibit the same general trend independently of the SES of the adopted children's biological parents. □

TABLE 1 SES of parents

Measure of SES	Groups of adopted children			
	B+/A+ n=10	B+/A- n=8	B-/A- n=10	B-/A+ n=10
Biological parents				
NYS	15.3 (1.4)	14.8 (1.2)	6.7 (1.1)	6.6 (0.8)
	14-18	13.5-17.5	5-5.9	5-7
OS	Student	Student	Worker	Worker
	Physician	Physician	Diverse	Farmworker
		Senior executive	unskilled	Diverse unskilled
Adoptive parents				
NYS	16.8 (2.4)	6.8 (1.2)	6.3 (0.7)	17.3 (1.3)
	14.5-23	5-8	5-7	15-19
OS	Physician	Worker	Worker	Physician
	Senior executive	Small farmer	Small farmer	Senior executive
	Professor	Diverse unskilled	Diverse unskilled	Professor

NYS, number of years of schooling—mid-parent value, standard deviation and range. OS, occupational status of biological and adoptive parents. Groups were composed of children who fulfilled the following conditions: 1, relinquished at birth; 2, placed for adoption at no more than 6 months of age; 3a, born to two high SES biological parents B+; 4a, adopted by two high (A+; the B+/A+ group) or two low (A-; B+/A-) SES parents; or; 3b, born to two low SES biological parents B-; 4b, adopted by two high (B-/A+) or low SES parents (B-/A-). The mean NYS for both biological and adoptive parents corresponds to the minimal NYS between the first year of mandatory education and the level of education attained when leaving the school system (repeated years were not counted). NYS ranged from a maximum of 9 for low SES parents (A- and B-) and a minimum of 13 for the high SES (B+ and A+). Occupations were defined as high or low according to the French Occupational Status Classification Scale¹¹. Adopted children B+/A- and B-/A+ changed postnatal environment, whereas control group children B+/A+ and B-/A- were adopted by parents having an SES identical to the SES of their biological parents.

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An essential role for postsynaptic calmodulin and protein kinase activity in long-term potentiation

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THE phenomenon of long-term potentiation (LTP), a long lasting increase in the strength of synaptic transmission which is due to brief, repetitive activation of excitatory afferent fibres, is one of the most striking examples of synaptic plasticity in the mammalian brain. In the CA1 region of the hippocampus, the induction of LTP requires activation of NMDA (*N*-methyl-D-aspartate) receptors by synaptically released glutamate¹ with concomitant postsynaptic membrane depolarization^{2–5}. This relieves the voltage-dependent magnesium block of the NMDA-receptor ion channel^{6,7}, allowing calcium to flow into the dendritic spine^{8–10}. Although calcium has been shown to be a necessary trigger for LTP (refs 11, 12), little is known about the immediate biochemical processes that are activated by calcium and are responsible for LTP. The most attractive candidates have been calcium/calmodulin-dependent protein kinase II (CaM-KII) (refs 13–16), protein kinase C (refs 17–19), and the calcium-dependent protease, calpain²⁰. Extracellular application of protein kinase inhibitors to the hippocampal slice preparation blocks the induction of LTP (refs 21–23) but it is unclear whether this is due to a pre- and/or postsynaptic action. We have found that intracellular injection into CA1 pyramidal cells of the protein kinase inhibitor H-7, or of the calmodulin antagonist calmidazolium, blocks LTP. Furthermore, LTP is blocked by the injection of synthetic peptides that are potent calmodulin antagonists and inhibit CaM-KII auto- and substrate phosphorylation. These findings demonstrate that in the postsynaptic cell both activation of calmodulin and kinase activity are required for the generation of LTP, and focus further attention on the potential role of CaM-KII in LTP.

Figure 1a shows a comparison between the tetanus-induced potentiation of the intracellular excitatory postsynaptic potential (e.p.s.p.) in cells recorded with electrodes filled with normal control recording solution, and cells recorded with electrodes which contained in addition 20 mM H-7, a potent but non-specific kinase inhibitor²⁴ (see Table 1). Control cells showed robust potentiation at 60 min, whereas H-7 filled cells showed a decremental potentiation which returned to baseline after ~30–40 min. The time course of this potentiation is similar to that seen following extracellular application of H-7 (ref. 21). Figure 1b shows that the magnitude of the LTP induced in the

cells surrounding those which were impaled, as measured by monitoring field e.p.s.ps, was similar in the two populations of hippocampal slices.

Both CaM-KII and protein kinase C (PKC) have been proposed to play a role in LTP. A number of calmodulin (CaM) antagonists have been reported to block LTP, following bath application, to the entire hippocampal slice^{22,25–27}. As was the case with H-7, however, it is unclear whether these compounds acted pre- and/or postsynaptically. To test whether activation of calmodulin in the postsynaptic cell is required for LTP, we recorded from cells with electrodes filled with 0.5 mM calmidazolium. Calmidazolium blocked LTP (Fig. 1c) in a manner similar to that seen when cells were filled with H-7. This could not be attributed to the effects of dimethyl sulphoxide (DMSO), the solvent for calmidazolium, as the control cells recorded with electrodes filled with 1% DMSO exhibited LTP. Figure 1d shows that the magnitude and time course of the LTP induced in the two populations of slices were again very similar. This set of experiments suggests that activation of CaM within the postsynaptic cell is required for the generation of LTP.

Calmidazolium, however, like other calmodulin antagonists tested on LTP, has actions in addition to its ability to antagonize calmodulin^{28,29}. Furthermore, both H-7 and calmidazolium could slowly cross neuronal membranes, making it difficult to rule out absolutely effects on adjacent presynaptic terminals. It was therefore important to examine further the role of postsynaptic calmodulin in LTP using inhibitors of calmodulin that are more specific and less membrane-permeable. Recently a synthetic CaM-binding peptide (CBP), the sequence of which is based on the CaM-binding domain of CaM-KII, has been shown to bind potently to CaM and inhibit the CaM-dependent activation of CaM-KII (Table 1)³⁰. In an initial set of experiments, we compared the effects on LTP of intracellularly applied CBP and CTP₂, a control peptide that shares sequence homology with CBP and has a similar isoelectric point, but is not a CaM antagonist (Table 1). For all experiments involving intracellular application of peptides, the same sample of peptide used for electrophysiological experiments was also tested biochemically, as described in Table 1. Figure 2a shows that LTP was blocked in cells recorded with electrodes containing CBP, whereas CTP₂ had no apparent effect on LTP. The simultaneously recorded field e.p.s.ps are plotted in Fig. 2b and demonstrate that there was no observable difference in the LTP induced in the two populations of hippocampal slices.

If the three N-terminal amino acids are removed from CBP, a peptide is obtained (CBP₋₃) which has the same CaM antagonistic activity but, unlike CBP, does not block Ca²⁺/CaM-independent substrate phosphorylation by previously autophosphorylated CaM-KII (Table 1)³⁰. Thus, if CBP₋₃ differed from

TABLE 1 Inhibition of substrate phosphorylation

	CaM-KII		PKC
	Ca ²⁺ /CaM-dependent IC ₅₀ (μM)	Ca ²⁺ /CaM-independent IC ₅₀ (μM)	IC ₅₀ (μM)
CBP	0.090	2.0	>150*
CBP ₋₃	0.090	>150*	>150*
CTP ₂	170	>2,000*	—
H-7	20	32	8

The substrates for the two forms of CaM-KII were synapsin I (4–50 μM per assay) or the synthetic peptide MHRQETV/DG-amide (single-letter amino-acid code; 15 μM); for PKC the substrate was syntide (15 μM) (ref. 30). IC₅₀ values were determined as described previously³⁰. The Ca/CaM-independent form of CaM-KII was prepared by autophosphorylation as described³⁰. PKC was purified and assayed as described³⁸, except that chromatography on protamine-agarose was omitted. Final ATP concentration in all assays was 15 μM. The sequence of CBP is MHRQETVDCLKKFNARRKLKGAILTTMLA, that of CBP₋₃ is QETVDCLKKFNARRKLKGAILTTMLA, and that of CTP₂ is ILTTLATRNFGSGGK. CTP₂ was a gift from Ruthann Masaracha.

* No inhibition of kinase activity was observed at this concentration.

CBP in its ability to block LTP, it would strongly suggest that the blockade of LTP by CBP requires the inhibition of CaM-independent CaM-KII activity in addition to its CaM antagonist activity. Figure 2c and d demonstrates that the blockade of LTP by CBP₋₃ is indistinguishable from the blockade by CBP. This result indicates that the ability of the two peptides to block LTP must be attributed to the inhibition of CaM. Taken together, the results using electrodes filled with calmidazolium, CBP, CBP₋₃ or CTP₂ indicate that the activation of postsynaptic CaM is a critical requirement for the generation of LTP. In addition, our results clearly demonstrate the power of using inhibitory peptides, synthesized from primary sequence data, to study synaptic physiology and intracellular biochemistry in mammalian brain tissue.

To test the possibility that the peptides may have exerted their effect by a non-specific depressant effect on synaptic transmission we examined the effects of CBP on LTP evoked in one pathway while simultaneously recording e.p.s.ps in response to an independent control pathway. Figure 3a shows that CBP blocked LTP in the intracellularly recorded cells, but had essen-

tially no effect on the control e.p.s.ps. The field e.p.s.ps recorded in response to the two pathways are plotted in Fig. 3b which shows that LTP was induced in the tetanized pathway, whereas synaptic transmission in the control pathway was stable. Thus CBP has no observable effects on normal synaptic transmission.

The biochemical mechanisms underlying LTP in the CA1 region of the hippocampus have generated much research and discussion. The data presented here indicate that activation of postsynaptic CaM, presumably by the rise in dendritic spine Ca^{2+} initiated by tetanic stimulation, is a requisite step in the induction of LTP. Furthermore, we have demonstrated that postsynaptic kinase activity is also required for LTP. The simplest explanation for these findings is that LTP requires CaM-dependent activation of CaM-KII. The potential involvement of other kinases or CaM-dependent processes cannot be ruled out. The fact that CBP and CBP₋₃ do not inhibit PKC activity *in vitro* (Table 1), however, suggests that PKC alone cannot be responsible for LTP.

CaM-KII is a particularly favourable candidate to play a critical role in LTP because it makes up 20–40% of the protein

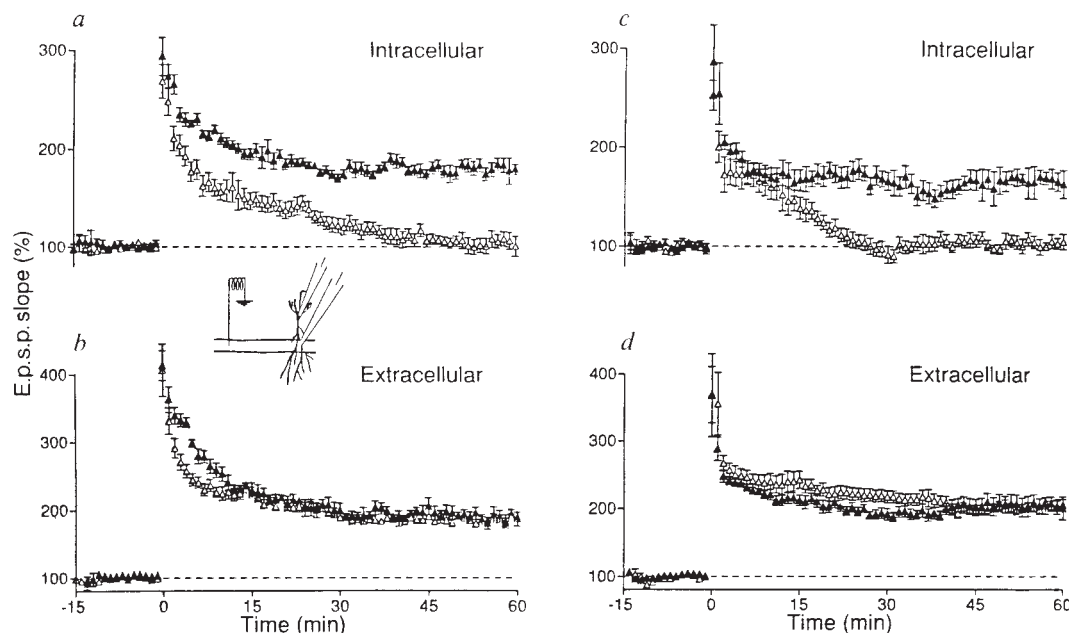


FIG. 1 Intracellular application of H-7, a protein kinase inhibitor, or calmidazolium, a CaM antagonist, blocks LTP. a, Comparison of changes in the magnitude of the initial slope of populations of cells recorded with microelectrodes containing either (Δ) H-7 (20 mM in 3 M CsCl; $n=8$) or ($\blacktriangle$) CsCl (3 M; $n=6$) following LTP-inducing stimuli. Inset, a diagram of the position of the stimulating and recording microelectrodes. b, Magnitude and time course of LTP induced in the two populations of slices as measured by changes in the initial slope of simultaneously recorded field e.p.s.ps. For this and all subsequent figures, symbols represent the mean $\pm$ s.e.m. c, Comparison of the e.p.s.p. initial slope following LTP-inducing tetani in populations of cell recorded with microelectrodes containing either (Δ) calmidazolium (0.5 mM in 1% DMSO; $n=5$) or ($\blacktriangle$) 1% DMSO alone ($n=5$). d, The initial slope of the field e.p.s.ps is plotted and demonstrates that the magnitude of LTP induced in the two populations of slices was the same.

METHODS. Hippocampal slices were prepared as previously described³⁹. All experiments involved stimulation of the Schaffer collateral/commissural afferents at 0.1 Hz using a bipolar, stainless steel stimulating microelectrode placed in stratum radiatum. The medium in all experiments was (mM): NaCl (119), KCl (4), MgSO_4 (4), CaCl_2 (4), NaH_2PO_4 (1), NaHCO_3 (26), dextrose (11), picrotoxin (0.1). A surgical cut was made between CA1 and CA3 regions to prevent epileptiform discharges. Intracellular recordings were made in stratum pyramidale using microelectrodes (40–70 M Ω) filled with 3 M CsCl. To monitor the occurrence and magnitude of LTP in each hippocampal slice, field e.p.s.ps were simultaneously recorded in stratum radiatum using a low resistance (2–6 M Ω) microelectrode containing 3 M NaCl. During the course of each experiment, a cell was impaled and after a 10–20 min waiting period,

a 10–20 min stable baseline was obtained. Tetanic stimulation consisted of two 1 s tetani at 100 Hz separated by 20 s and was given at 1.5 times the control stimulation strength. Only those experiments in which the field e.p.s.p. initial slope remained greater than 120% of its control value at 60 min after the tetanus, were included in the data analysis (~85% of experiments). At all times during the experiment, except during the tetani, hyperpolarizing current (d.c.) was passed so that the membrane potentials of the cells were kept at between -70 and -90 mV. This routinely prevented the generation of Ca^{2+} spikes or other voltage-dependent events which might result in the pairing of membrane depolarization with synaptic stimulation, the minimal requirements for the induction of LTP. To ensure that during the tetani adequate depolarization was achieved to induce LTP, the hyperpolarizing holding current was removed. All data were digitized on line using an IBM AT-compatible microcomputer. The maximum initial slope of each e.p.s.p. was calculated using a modified version of pClamp 4 (Axon Instruments). Each slope measurement in an individual experiment was normalized to the average value of all points on the baseline (at least 10 min before LTP induction). Individual experiments were then aligned with respect to the time of tetanic stimulation and divided into 60s bins, each of which was averaged to give the graphs illustrated. H-7 was obtained from Seikagaku and calmidazolium from Sigma. Peptides were synthesized and analysed as described³⁰. The concentration of these compounds within the recorded cells is unknown. Hyperpolarizing current (d.c.) would be expected to retard the diffusion of these basic molecules into the cell, in part accounting for the requirement of concentrations within the recording microelectrode that are ~1,000-fold higher than the respective IC_{50} values.

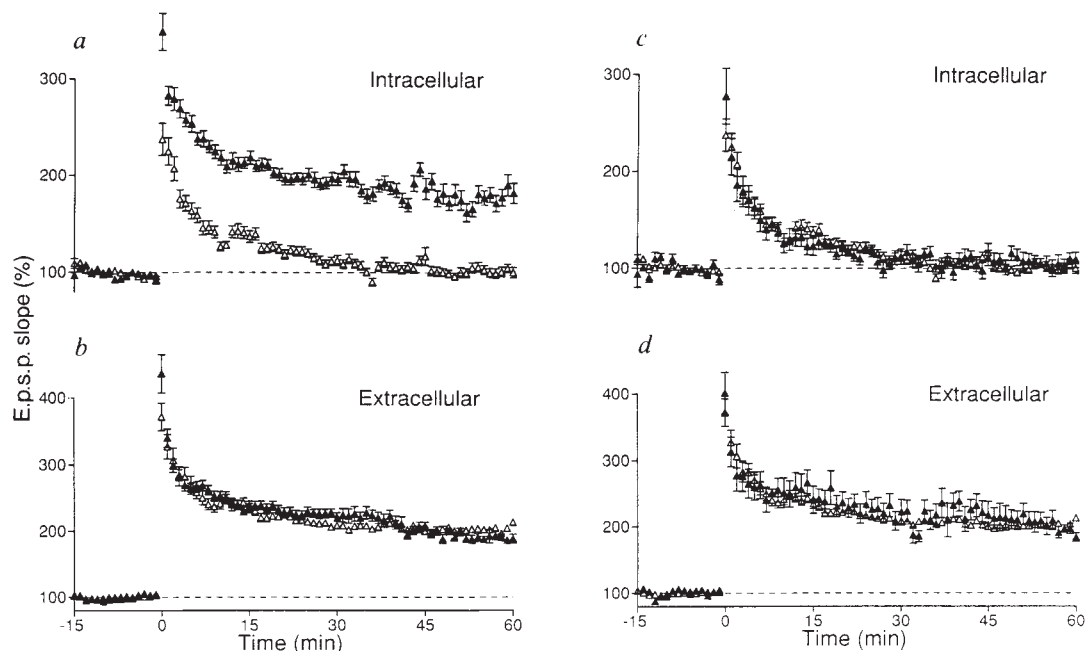


FIG. 2 Effect of intracellular application of the CaM-binding peptide (CBP) on LTP. *a*, The magnitude of the initial e.p.s.p. slope in populations of cells recorded with microelectrodes containing either (Δ) CBP ($190 \mu\text{M}$; $n=11$) or ($\blacktriangle$) the control peptide CTP₂ ($190 \mu\text{M}$; $n=8$). *b*, The initial field e.p.s.p. slope recorded in the two populations of slices demonstrating that the LTP was essentially identical in the two populations. *c*, The peptide, CBP₋₃, which

inhibits CaM activity but does not block Ca/CaM-independent CaM-KII substrate phosphorylation, has the same ability to block LTP as does CBP. Microelectrodes contained $190 \mu\text{M}$ CBP₋₃ ($n=6$). The data for the CBP-filled cells are the same as shown in *a*. *d*, LTP generated in the field potential is very similar for the two populations of slices.

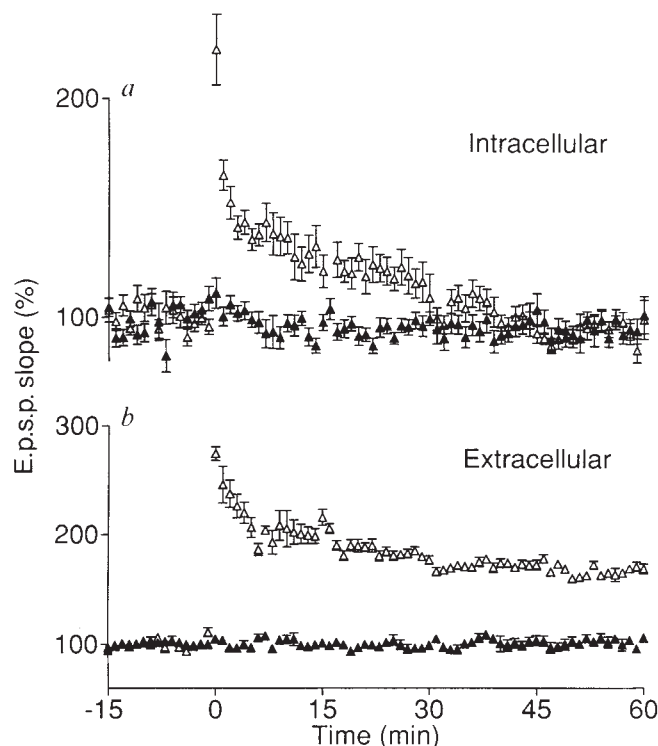


FIG. 3 CBP blocks LTP but does not affect normal synaptic transmission. Two independent pathways S1 (Δ) and S2 ($\blacktriangle$) synapsing on the same cell (*a*) or population of cells (*b*) were alternatively stimulated at 0.1 Hz. Standard LTP-inducing tetani were applied to the S1 pathway while stimulation of the S2 pathway was halted. Graphs are a summary of 4 experiments. No difference was observed when cells were recorded with microelectrodes containing 1.9 mM CBP ($n=2$) or $190 \mu\text{M}$ CBP ($n=2$).

found in isolated postsynaptic densities^{13,14} and should have ready access to the rise in Ca^{2+} and subsequent activation of CaM due to NMDA receptor activation. It is also in an ideal position to modify postsynaptic glutamate receptors. Biochemical studies have demonstrated that following CaM-dependent autophosphorylation, CaM-KII no longer requires CaM to maintain its kinase activity^{15,31}. It has recently been proposed that persistent kinase activity is required for the maintenance of LTP^{21,23}. As has been predicted from modelling of the biochemical properties of CaM-KII, the initial activation of CaM by postsynaptic Ca^{2+} may permit CaM-KII to become constitutively active and serve as a mechanism for maintaining increased synaptic strength^{15,16}. The simplest hypothesis to account for the increase in synaptic strength during LTP is that activation of CaM, kinase activity and perhaps as yet unknown biochemical processes, somehow modify the number and/or properties of the non-NMDA receptors which generate the e.p.s.p. at these synapses^{20,32-34} although more complicated events cannot be ruled out³⁵⁻³⁷. □

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Thymic cortical epithelial cells lack full capacity for antigen presentation

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SEVERAL recent studies have suggested that interactions between thymocytes and thymic stromal cells are essential for the development and elimination of antigen-reactive T lymphocytes¹⁻⁴. It is important, therefore, to characterize the stromal cells involved in presentation of antigen in the thymus. In a previous report, we demonstrated, using T-cell hybridomas, that three distinct types of antigen presenting cells in the thymus (cortical epithelial cells, macrophages, and dendritic cells) constitutively expressed self haemoglobin/Ia complexes⁵. Here we report that one of these cell types, the cortical epithelial cell, does not induce stimulation of T-lymphocyte clones even though the antigen/Ia complex required for antigen-specific recognition is present. This lack of response occurs with both T_{H1} and T_{H2} clones. Responsiveness of the T_{H2} clone can be restored by adding the murine lymphokine interleukin-1 β to the culture system.

T-cell hybridomas are known to be stimulated by the engagement of their T-cell receptor (TCR) with antigen/Ia complexes. By contrast, freshly isolated T-cell clones require in addition the presence of one or more secondary molecules, such as adhesion or co-stimulatory proteins, for T-cell stimulation to occur. To fully define the antigen presenting capabilities of thymic stromal cells, it is important to discover whether they express these additional molecules. For this investigation, two haemoglobin (Hb)-specific, CD4⁺, I-E^k-restricted T-cell clones, PL17 and HS8 were used. On stimulation, these T-cell clones secreted interleukin-2 (IL-2) and γ -interferon but not IL-4, and were therefore classified as belonging to the T_{H1} subset of murine helper T-cell clones⁶. As observed with murine Hb-specific hybridomas, the Hb-specific T-cell clones responded to splenic antigen presenting cells (APCs) directly after removal of the spleens from the mouse, in the absence of any exogenous antigen (Fig. 1).

We have previously demonstrated that three types of APCs in the thymus, cortical epithelial cells, macrophages, and dendritic cells, can stimulate Hb-specific T-cell hybridomas⁵. The Hb-specific T-cell clones were tested, therefore, for reactivity towards these three types of APCs. The results (Fig. 2a) clearly show that thymic nurse cells (TNC), the *in vitro* correlate of cortical epithelial cells⁷⁻⁹, did not stimulate the HS8 clone to proliferate, even though the TNCs had sufficient Hb/Ia complexes to stimulate the YO1.6 hybridoma (Fig. 2a, top). The other two populations of APCs, the M ϕ /DC and the T-ROS

(a representative sample of Ia⁻ macrophages and Ia⁺ dendritic cells, which when isolated have rosetted thymocytes surrounding them) were both fully capable of stimulating the HS8 clone (Fig. 2a, bottom). Identical results were seen using the PL17 clone (data not shown). As shown in Fig. 2b, equivalent results, with a limited-response to the thymic cortical epithelial cell population, were found when a hen egg-white lysozyme (HEL)-specific T_{H2} clone, CIB4 (ref. 10), was used as the responding cell. No

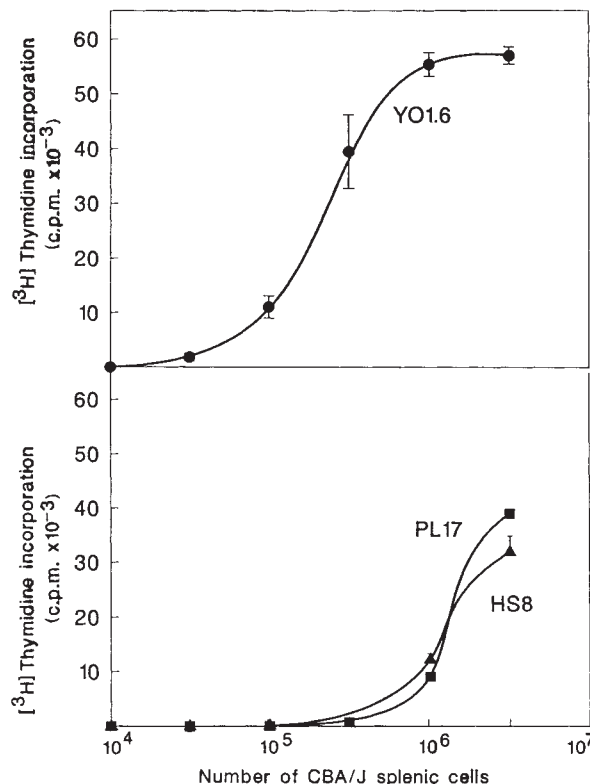
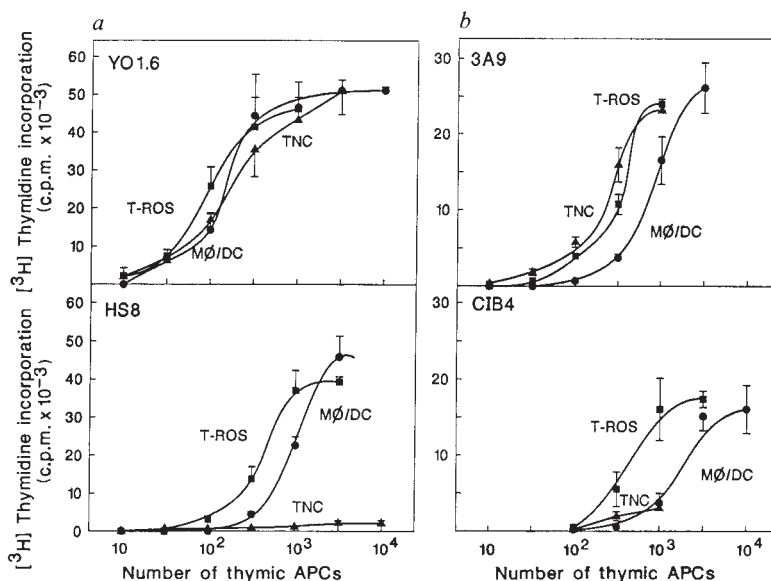


FIG. 1 Stimulation of the Hb-specific YO1.6 hybridoma (top) and the Hb-specific PL17/HS8 clones (bottom) by CBA/J splenic APCs. It should be noted that the PL17 and HS8 clones recognize the same peptide antigen Hb ^{β} minor (62-76) as the YO1.6 hybridoma, and are approximately tenfold less sensitive to the *in vitro* Hb/Ia complexes than the YO1.6 hybridoma. No stimulation of either the YO1.6 hybridoma or the PL17/HS8 clones was seen when splenic cells isolated from mice with the non-stimulatory Hb haplotype (Hbb^s, H-2^k) were used.

METHODS. The stimulation of the Hb-specific T-cell clones was quantitated by measuring the [³H] thymidine incorporation of the proliferating T-cell clone (1×10^4 per well) in response to irradiated CBA/J splenic APCs (3,000 rads). [³H] Thymidine was added at 72 h and the cells were collected 24 h later. The hybridoma stimulation assay was as described¹⁶, with the exception that a single cell suspension of irradiated splenic cells was used as the APC population. No exogenous antigen was added to the culture system. The specificity of the YO1.6 hybridoma is as described¹⁶. The I-E^k-restricted Hb-specific T-cell clones were generated by immunizing CE/J mice (Hbb^s) with 20 μ g hemolysate from CBA/J mice (Hbb^d) emulsified in complete Freund's adjuvant (Difco H37Ra). Seven days later the draining lymph nodes were removed, a single cell-suspension was prepared and incubated in upright T-25 flasks (Corning) at 1×10^7 cells ml⁻¹ with 100 μ g ml⁻¹ CBA/J hemolysate in 5 ml RPMI 1640 supplemented with 10% horse serum, 2 mM L-glutamine and 50 μ g ml⁻¹ gentamicin. Four days later, the T-cell blasts were isolated on a Ficoll-Hypaque gradient (Pharmacia) and recultured at 1×10^5 cells ml⁻¹ with irradiated (3,000 rads) CE/J spleen filler cells (5×10^6 ml⁻¹) in media containing 50 μ M 2-mercaptoethanol and 10% FCS instead of horse serum. Ten days later, the viable cells were recovered and recultured with 5×10^6 ml⁻¹ irradiated CBA/J filler cells at 1×10^5 cells ml⁻¹. This bulk line was passed every 14 days on fresh CBA/J filler cells. At the end of the second biweekly cycle, the bulk line was subcloned in microtitre wells containing 20% rat con-canavalin A (con A) supernatant and 1×10^6 CBA/J filler cells per well. Positive wells were expanded on fresh filler cells and weaned off rat con A supernatant. These T-cell clones were maintained on a cycle of 4 day stimulation on CBA/J (Hbb^d) filler cells and 10 day rest on B10.BR/SgSnJ (Hbb^s) filler cells and 0.5% rat con A supernatant.

FIG. 2 Stimulation of antigen-specific T-cells by purified thymic APC populations. T-cells used in the assay were: *a* (top) Hb-specific YO1.6 hybridoma, and (bottom) Hb-specific HS8 T_{H1} clone; *b* (top) HEL-specific 3A9 hybridoma (ref. 17), and (bottom) HEL-specific CIB4 T_{H2} clone. The top panel of *a* reproduces our previous results⁵. The bottom panel indicates a lack of stimulation by TNC, in contrast to the normal level of stimulation induced using Mφ/DC or T-ROS. The top panel of *b* shows the stimulation of a T-cell hybridoma after pulsing the three thymic APC populations with the peptide HEL(46–61). In the bottom panel, the CIB4 clone was stimulated by Mφ/DC and T-ROS, but was only weakly stimulated by TNC. The results in *b* (bottom), rule out the possibility that previous thymic APC data showing non-stimulation by TNC were due to contamination by Mφ/DC or T-ROS cells. Because these cell types stimulate T-cell clones, this effect would have been observed if these cells were present in an impure TNC population.

METHODS. The stimulation assays were as described in Fig. 1 and all APCs were irradiated with 3,000 rads regardless of whether they were used for antigen presentation to hybrids or clones. Exogenous antigen was added to all culture wells, 31.6 μM Hb^{B_dminor}(62–76) to the YO1.6 and HS8 cultures, and 10 μM HEL(46–61) to the 3A9 and CIB4 cultures. In the absence of exogenous antigen, the minimum number of thymic APCs needed to stimulate the YO1.6 hybridoma was 3×10^3 total cells. As was noted in Fig. 1, the Hb-specific T-cell clones used in this study are about tenfold less responsive to *in vivo* Hb/Ia complexes. This fact means that at least 3×10^4 total cells per data point would be needed before the T-cell clones would stimulate a measurable response to *in vivo*



complexes on thymic APCs. This is an impractical number because of the requirement for large numbers of young thymuses, and all further experiments were performed, therefore, by adding exogenous Hb antigen. The thymic APC populations were isolated exactly as described⁵. The CIB4 clones were maintained as described¹⁸.

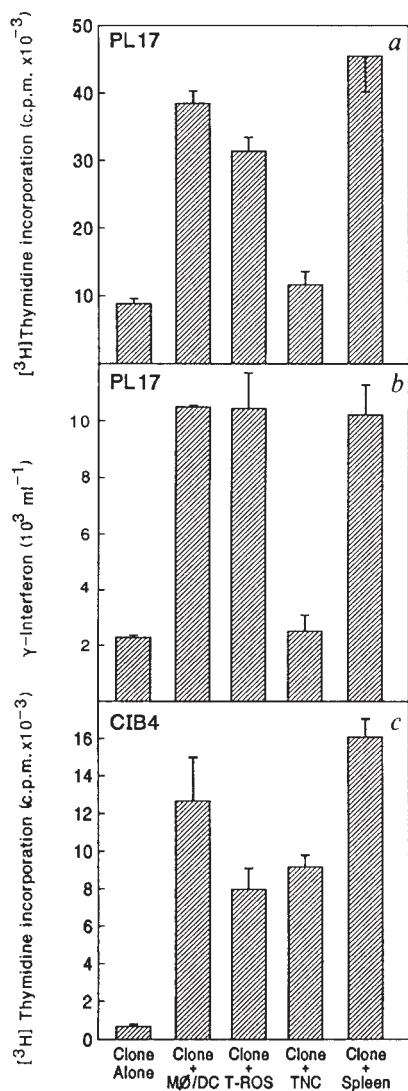


FIG. 3 Lymphokine secretion by T-cell clones stimulated by purified thymic APC populations. *a*, IL-2 secretion by the Hb-specific PL17 clone. *b*, γ-interferon secretion by the Hb-specific PL17 clone. *c*, IL-4 secretion by the HEL-specific CIB4 clone. Identical results for IL-2 secretion were seen using the HS8 Hb-specific T-cell clone. IL-2 and IL-4 were secreted by the respective clones in an APC dose dependent manner.

METHODS. T-cell clones were used at 1×10^5 per well. Supernatants for measurement of IL-2 and IL-4 were collected after 24 h stimulation, while the γ-interferon samples were collected after 72 h. Antigen and irradiated APCs were used as described above. For determination of IL-2 and IL-4 secretion, the APC concentrations were as follows: Mφ/DC, 1×10^4 per well; T-ROS, 3×10^3 per well; TNC, 3×10^3 per well; and spleen, 3×10^5 per well. For the γ-interferon determinations, the APC concentrations were: Mφ/DC, 3×10^4 per well; T-ROS, 1×10^4 per well; TNC, 1×10^4 per well; and spleen, 1×10^6 per well. No lymphokine secretion was detectable when the APC populations were tested in the absence of T-cell clones. The presence of IL-2 or IL-4 was determined by the stimulation of [³H] thymidine incorporation by the IL-2/IL-4 dependent cell line CTLL-2. The secretion of IL-2 versus IL-4 was determined by antibody blocking studies using the αIL-4 antibody 11B11 and the αIL-2R antibodies 7D4 and PC61 as described¹⁸. An ELISA assay was used to determine the levels of γ-interferon¹⁹.

additional proliferation by CIB4 was found in a second experiment, when a tenfold higher concentration of TNCs was added (data not shown).

One possible explanation for the non-responsiveness of our T-cell clones to the TNC population, is that the enzymatic digestion to which the TNCs are subjected during isolation is stripping off a co-stimulatory molecule. We therefore subjected splenic cells to exactly the same isolation procedure and then tested them for stimulation of T-cell clones. This enzymatically digested splenic cell preparation stimulated the T-cell clones equivalently to untreated spleen cells, ruling out this possibility (data not shown).

To further investigate the non-responsiveness to TNCs, we measured lymphokine secretion and observed an interesting difference between T_{H1} and T_{H2} clones. The Hb-specific T_{H1} clone did not secrete significant amounts of either IL-2 or γ-interferon in response to TNCs (Figs 3a, b), whereas the T_{H2} clone CIB4 secreted normal levels of IL-4 when subjected to the equivalent TNC population and the appropriate antigen (Fig. 3c). This is not surprising, as it has been previously demon-

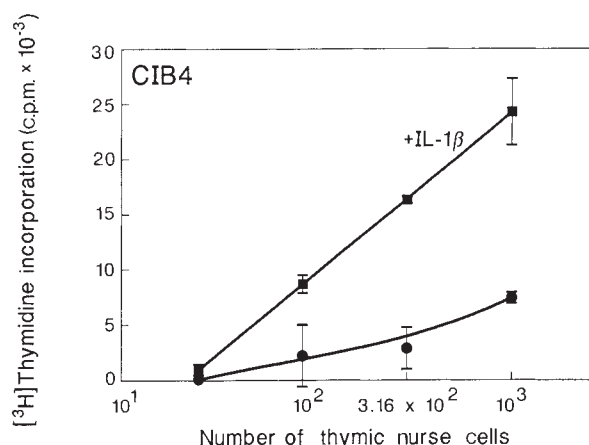


FIG. 4 Addition of the co-stimulator molecule rIL-1 β facilitates the antigen-presenting function of the TNC which can then stimulate the T_{H2} clone CIB4. TNCs were isolated as described previously⁵ and were irradiated with 3,000 rads. No proliferation is observed when the T-cell clones are eliminated from the TNC/rIL-1 β culture. Irradiation of the TNC population at lower levels (900 rads) still did not enable stimulatory antigen presentation by the TNC (data not shown). HEL(46-61) was used at 7.5 μ M and rIL-1 β was added at 940 μ g ml⁻¹. The proliferation assay was as described in Fig. 1.

strated that IL-4 production by T_{H2} clones, in contrast to IL-2 production in T_{H1} clones, requires only TCR occupancy for its elicitation¹¹. Currently, the importance of this differential lymphokine secretion in thymic T-cell development is unknown.

Our results point to the thymic cortical epithelial cell as the only thymic stromal cell with defective antigen presentation ability. In a previous study of thymic APCs, it was observed that TNCs did not stimulate sperm-whale myoglobin-specific T-cell clones either¹². These results did not establish whether the intravenously injected sperm-whale myoglobin actually reached the thymic cortex, or if it did, whether it was processed and presented in an immunogenic manner. As our previous and present results conclusively show that TNCs express Hb/Ia complexes, we conclude that these phenomena are due to the lack of a second signal required for stimulation of T-cell clones.

It has been suggested that delivery of an antigenic signal alone through the TCR $\alpha\beta$ induces clonal inactivation, whereas this same signal in the presence of a co-stimulator molecule or mitogenic signal will cause clonal activation^{13,14}. If non-responsiveness is due to the presence of the antigenic signal alone, then addition of the co-stimulatory signal to the TNC should enable antigen presentation resulting in clonal stimulation. We tested this possibility by adding recombinant interleukin-1 β (rIL-1 β), a known co-stimulator for T_{H2} clones, to the culture containing TNCs and the T_{H2} clone CIB4 (refs 10, 15). As postulated, the addition of rIL-1 β facilitated antigen presentation by the TNC, and subsequently, stimulation of the CIB4 clone (Fig. 4). Further experiments are required to determine if TNCs produce inhibitory factors or respond to rIL-1 β by making secondary molecules, in addition to their lack of co-stimulator molecules. We were not able to extend this line of experimentation to the T_{H1} clones as the identity of the T_{H1} co-stimulator molecule is currently unknown.

The results presented here are a direct demonstration that a class of thymic stromal antigen presenting cell lacks the full capacity to induce T-lymphocyte stimulation. The requirement for co-stimulator activity in the differentiation of thymocytes is unknown, and the response of our mature T-cell clones may not be identical to that of a developing thymocyte. Our data clearly show, however, that despite the presence of Hb/Ia complexes on their cell surface, thymic cortical epithelial cells are unable to carry out the entire process of T-lymphocyte stimulation. We conclude that co-stimulatory signals are important in thymic education. □

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Phenotypic differences between $\alpha\beta$ versus β T-cell receptor transgenic mice undergoing negative selection

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T-CELL differentiation in the thymus is thought to involve a progression from the CD4⁺CD8⁺ phenotype through CD4⁺CD8⁺ intermediates to mature CD4⁺ or CD8⁺ cells^{1–3}. There is evidence that during this process T cells bearing receptors potentially reactive to 'self' are deleted by a process termed 'negative selection'^{4–10}. One example of this process occurs in mice carrying polymorphic Mls antigens, against which a detectable proportion of T cells are autoreactive. These mice show clonal deletion of thymic and peripheral T-cell subsets that express the autoreactive V β 3 segment of the T-cell antigen receptor, but at most a two-fold depletion of thymic cells at the CD4⁺CD8⁺ stage^{5–7}. By contrast, transgenic mice bearing both α and β chain genes encoding autoreactive receptors recognizing other ligands, show severe depletion of CD4⁺CD8⁺ thymocytes as well^{8,9}, suggesting that negative selection occurs much earlier. We report here the Mls 2^a/3^a mediated elimination of T cells expressing a transgene encoded V β 3-segment, in T-cell receptor α/β and β -transgenic mice. Severe depletion of CD4⁺CD8⁺ thymocytes is seen only in the α/β chain transgenic mice, whereas both strains delete mature V β 3 bearing CD4⁺ and CD8⁺ T cells efficiently. We conclude that severe CD4⁺CD8⁺ thymocyte deletion in α/β transgenic mice results from the premature expression of both receptor chains, and does not reflect a difference in the timing or mechanism of negative selection for Mls antigens^{5–7} as against the allo- and MHC class I-restricted antigens used in the other studies^{8–9}.

We have produced lines of transgenic mice carrying either the rearranged T-cell receptor α -chain gene (refs 11, 12) or

β -chain gene¹³ from the T-helper hybridoma, 2B4 (ref. 14). The β chain of this receptor uses the $V_{\beta}3$ gene segment. Recently, it has been demonstrated that T-cell receptors using the $V_{\beta}3$ gene segment are intrinsically reactive to products of the $Mls-2^a$ and $Mls-3^a$ loci, particularly in combination with the $H-2^k$ or $H-2^d$ MHC types; consequently, T cells carrying such receptors are deleted in strains of mice expressing either of these unlinked Mls loci^{7,15,16}. Initial studies showed that many, but not all, of the transgenic mice carrying the 2B4 β construct had a severe depletion of peripheral T cells, particularly those of the $CD4^+$ subset. The marked variation between different 2B4 β transgenic mice was probably due to random segregation of the $Mls-2^a$ and $Mls-3^a$ genes.

We have analysed the T cells in these mice with a $V_{\beta}3$ -specific antibody (KJ25, ref. 7). Normal C57Bl/6 mice express $V_{\beta}3$ on ~3% of peripheral T cells (Fig. 1a). Of the 2B4 β transgenic mice, some show 70–90% $V_{\beta}3$ -positive lymph node T cells, whereas others have less than 10% $V_{\beta}3$ -positive T cells. Severe T-cell depletion and loss of $V_{\beta}3$ expressing cells occurs more often (but not exclusively) in 2B4 β transgenic mice expressing $H-2^k$ alleles at the MHC (two examples are shown in Fig. 1b).

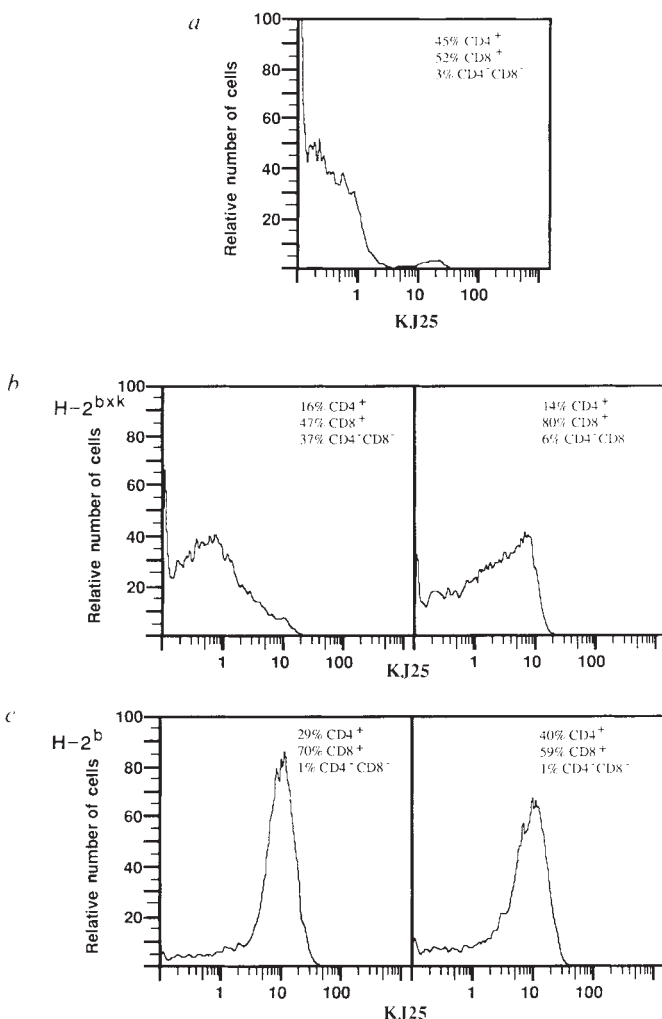


FIG. 1 Nylon-wool purified lymph node T cells were stained with the KJ25 (anti- $V_{\beta}3$) monoclonal antibody. a, Level of $V_{\beta}3$ expressed in a non-transgenic C57Bl/6 ($Mls-2^b$ and -3^b) mouse. b, Two 2B4 β transgenics of the $H-2^{k \times b}$ haplotype ($Mls-2^a$ and/or -3^a). c, Two 2B4 β transgenics of the $H-2^b$ haplotype ($Mls-2^a$ and/or -3^a). Percentage of $CD4^+$, $CD8^+$ and $CD4^+CD8^+$ peripheral T cells in each mouse is indicated (upper right). Cells were stained with biotinylated KJ25 (ref. 7) followed by avidin-FITC. Cells from each mouse (50,000) were analysed using an Epics C flow cytometer as previously described²³.

Transgenic mice of the $H-2^b$ haplotype showed much more variability with some lineages having only minor effects such as a decrease in $CD4^+$ T cells and a slight overall reduction in $V_{\beta}3$ expressing cells (Fig. 1c). The most severe cases of depletion (Fig. 1b, left) show a significant increase in $CD4^+CD8^-$ T cells.

Backcross analysis supports the explanation that the presence and independent segregation of the $Mls-2^a$ and $Mls-3^a$ genes¹⁷ is responsible for the variation seen among 2B4 β transgenic mice of the same $H-2$ haplotype.

To determine the stage at which T-cell depletion could first be observed, we compared thymocytes from transgenic mice which had deleted $V_{\beta}3$ -positive T cells in the periphery with those which had not. Thymocytes were analysed with anti- $CD4$ and anti- $CD8$ antibodies by FACS. Figure 2a shows the staining pattern of a normal thymus. 2B4 β $H-2^{k \times b}$ transgenic mice which do not show the $V_{\beta}3$ specific T-cell depletion, have a similar pattern of staining, with the exception of a 3-fold increase in $CD4^+CD8^-$ thymocytes and slight increases in (twofold) $CD4^+CD8^+$ and $CD4^-CD8^+$ cells (Figs 2b and 3).

By contrast, staining of thymocytes from an $H-2^{k \times b}$ β -transgenic mouse which has deleted $V_{\beta}3$ positive peripheral T cells shows an almost complete loss of $CD4^+CD8^-$ thymocytes, and a slight (twofold) decrease in absolute numbers of $CD4^+CD8^+$ thymocytes (Figs 2c and 3). As the majority of $CD3^+$ thymocytes in these mice express the 2B4 β -chain on their surface (data not shown), it appears that negative selection by $Mls-2^a$ and/or $Mls-3^a$ prevents maturation of immature $CD4^+CD8^+$ cells into brightly staining 2B4 β -positive $CD4^+CD8^-$ cells in the thymus.

These data correspond well with studies of the deletion of potentially self-reactive endogenous MHC class II-restricted T-cell receptors recognizing Mls antigens^{4-7,18}. Thymic staining in our 2B4 α/β transgenic mice, however, resembles that seen in mice expressing high levels of a self-reactive class I restricted receptor, with severe depletion of $CD4^+CD8^+$ thymocytes⁸⁻⁹ (summarized in Fig. 3). These thymuses show a 40-fold reduction in total cell numbers and, of the 2–3% of thymocytes which remain, most are $CD4^-CD8^-$ and only ~20% are $CD4^+CD8^+$.

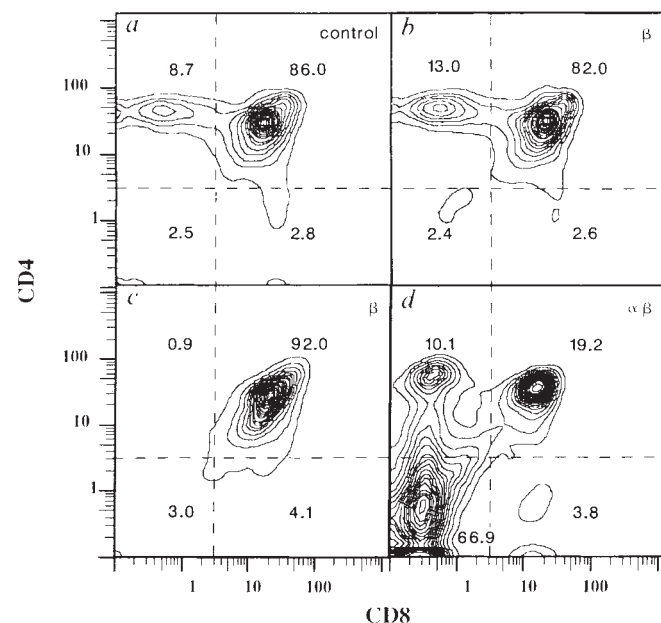


FIG. 2 Thymocytes from the following mice were stained with anti- $CD4$ versus anti- $CD8$ antibodies: a, non-transgenic ($H-2^{k \times b}$) control; b, 2B4 β transgenic, non- $V_{\beta}3$ -deleting, $H-2^{k \times b}$; c, 2B4 β transgenic, $V_{\beta}3$ -deleting, $H-2^{k \times b}$; d, 2B4 $\alpha\beta$ transgenic, $V_{\beta}3$ -deleting, $H-2^{k \times b}$. For each profile, the percentage of cells in each compartment is indicated. The thymus of the mouse shown in d ($\alpha\beta$, $V_{\beta}3$ -deleting, $H-2^{k \times b}$) had 40-fold fewer cells than a normal thymus. Cells were stained with directly conjugated anti- $CD4$ -PE (GK1.5, ref. 24, Becton-Dickinson) and anti- $CD8$ -FITC (53-6.7, ref. 25, Becton-Dickinson). Cells from each mouse (30–50,000) were analysed on a dual-laser FACS IV flow cytometer (Becton-Dickinson), as described²⁶.

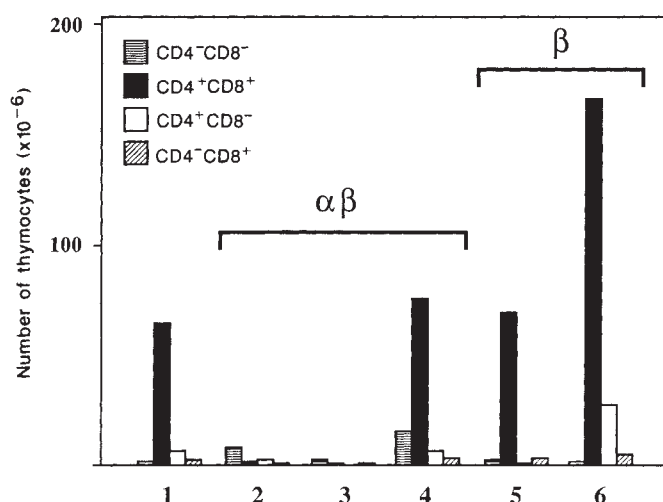


FIG. 3 The effects of negative selection on thymocytes of $\alpha\beta$ versus β -transgenic mice are shown graphically. The total number of thymocytes of each subset (CD4⁻CD8⁻, CD4⁺CD8⁺, CD4⁺CD8⁻, CD4⁻CD8⁺) are indicated for 1, non-transgenic control; 2, anti-(D^b+H-Y) $\alpha\beta$ transgenic⁸; 3, anti-(H-2^k+Mls-2^a and/or -3^a) $\alpha\beta$ transgenic; 4, non-V_β3-deleting $\alpha\beta$ transgenic; 5, anti-(H-2^k+Mls-2^a and/or -3^a) β -transgenic; 6, non-V_β3-deleting β -transgenic.

(Fig. 2d). A small number of CD4⁺CD8⁻ cells remain, but none of these are V_β3-positive.

These data suggest that severe CD4⁺CD8⁺ T-cell depletion results from expression in transgenic mice of high levels of both α - and β -chains of a given T-cell receptor heterodimer on most thymocytes. One general feature of α/β transgenic mice is the proportion of T-cell receptor positive cells in the thymus. In a normal mouse thymus, 50–60% of CD4⁺CD8⁺ cells express a heterogeneous distribution of low levels of the T-cell receptor/CD3 complex¹⁹. After staining of total thymocytes of a non-transgenic littermate (H-2^{kxb}, Mls-2^b and Mls-3^b) with anti-CD3, these CD3 weak-positive (+/-) cells can be clearly distinguished from the CD3⁻ thymocytes (see Fig. 4a, top panel). In a 2B4 β transgenic mouse the fraction of CD3⁺ thymocytes remains about the same (Fig. 4a, centre panel). By contrast, we find that in the α/β transgenic mice, over 95% of the thymocytes are positive for CD3. Furthermore, the level of T-cell receptor/CD3 on these CD3⁺ cells is skewed towards the highest level seen on CD4⁺CD8⁺ thymocytes in non-transgenic or 2B4 β transgenic mice (Fig. 4a bottom panel; also noted by Kiselow *et al.*⁸). Staining of thymocytes with KJ25 supports these observations, demonstrating that the significant increase in CD3-positive cells correlates with transgene expression (data not shown). These data suggest that the CD4⁺CD8⁺ thymocyte pool in α/β transgenic mice contains a disproportionately large fraction of cells with a more mature phenotype.

Staining of transgenic thymocytes with anti-CD5 antibodies reinforces the notion that the α/β transgenic thymus contains a higher proportion of mature cells (Fig. 4b). In a normal mouse thymus, as well as that of the β -transgenic mouse, the brightly stained CD5⁺ cells include all of the mature CD4⁺CD8⁻ cells as well as a small proportion of the CD4⁺CD8⁺ cells (data not shown). In the α/β transgenic mice, however, all of the CD4⁺CD8⁺ cells stain brightly with the anti-CD5 antibody (Fig. 4b, bottom panel). As this degree of CD5 staining corresponds to the levels of CD5 seen on mature peripheral T cells, we suggest that T cells in the α/β transgenic mice may be undergoing accelerated differentiation. This assertion is also supported by the earlier than normal appearance of α/β T-cell receptors in fetal thymocytes of α/β or α -only transgenic mice (Fazekas de St. Groth *et al.*, in preparation).

Our experiments show that the apparent difference in the timing of negative selection in the earlier experiments on trans-

genic mice^{8,9} and on tolerance to Mls⁵⁻⁷, cannot be due to differences in tolerance to class I as opposed to class II antigens, as we were able to reproduce both effects on thymic ontogeny in experiments on tolerance to class II alone. Similar observations have recently been reported by Pircher *et al.* in an analysis of negative selection of a V_β8.1-positive Mls^a-reactive β -chain in T-cell receptor β -chain transgenic mice¹⁰.

We suggest that the severe depletion of thymocytes in α/β transgenic mice results instead from accelerated maturation of CD4⁺CD8⁺ thymocytes, due to early co-expression of the α and β genes. This results in an increased proportion of cells in this compartment that are susceptible to negative selection, which has been previously shown to occur at this stage^{8,9,18,20,21}. A similar explanation has been suggested by Kiselow *et al.*⁸, and is consistent with the identification of two distinct CD4⁺CD8⁺ cell populations, which differ in their responses to anti-T-cell receptor versus anti-CD3 antibody binding²².

An alternative explanation for the differences in negative selection between $\alpha\beta$ - and β -only-transgenic mice might be the heterogeneity of the α -chains paired with the 2B4 β -chain in the β -transgenic mice, which could generate some receptors which are non-reactive to the Mls antigens. This is hard to reconcile, however, with the efficient deletion of V_β3-positive mature T cells in these mice.

One minor difference between our results and those of Kiselow *et al.*⁸ is that we do not observe self-reactive transgenic bearing T cells 'escaping' from negative selection into the periphery with low accessory molecule expression (in their case CD8); rather, we find cells with normal levels of CD4, but a down-regulation of V_β3. Thus, there may be differences between CD4⁺ and CD8⁺ T cells; alternatively, this could be due to differences in affinity between the particular self-ligand/MHC complexes involved and the T-cell receptor targets. □

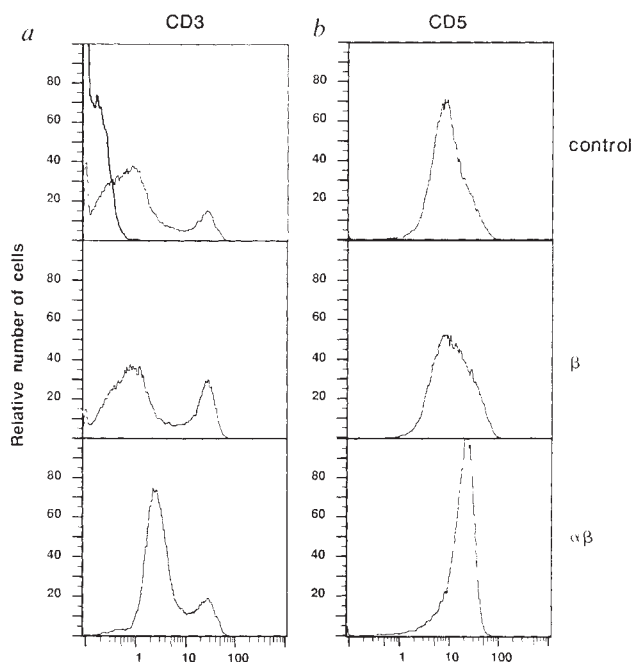


FIG. 4 Thymocytes of transgenic mice were stained with anti-CD3 and anti-CD5. For each antibody, the staining profiles of three mice are shown: a non-transgenic control (top panel), a β -transgenic (centre panel), and an $\alpha\beta$ transgenic (bottom panel), all H-2^{kxb} and lacking the V_β3-deleting alleles. a, Staining with anti-CD3 (500A2, ref. 27), followed by anti-hamster-FITC (Caltag). The bold line on the first profile shows the staining with anti-hamster-FITC alone. b, Staining with anti-CD5 (53-7.3, ref. 25) directly coupled to allophycocyanin (Biomedia). Cells from each mouse (30–50,000) were analysed on a dual laser FACS IV flow cytometer (Becton-Dickinson) as described²⁶.

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T-cell receptor δ -chain can substitute for α to form a $\beta\delta$ heterodimer

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SPECIFIC monoclonal antibodies have made possible the identification of two T-cell antigen receptor (TCR) heterodimers, $\alpha\beta$ TCR (refs 1–3) and $\gamma\delta$ TCR (ref. 4). Formation of these receptors is largely separated by the preferential pairing of α -TCR with β and γ -TCR with δ (refs 5 & 6), the sequential rearrangement and expression of the TCR loci during thymic development^{7–10} and the deletion of the δ -loci either prior to or concomitant with α -rearrangement in $\alpha\beta$ TCR cells^{11–13}. Here we show that δ -TCR

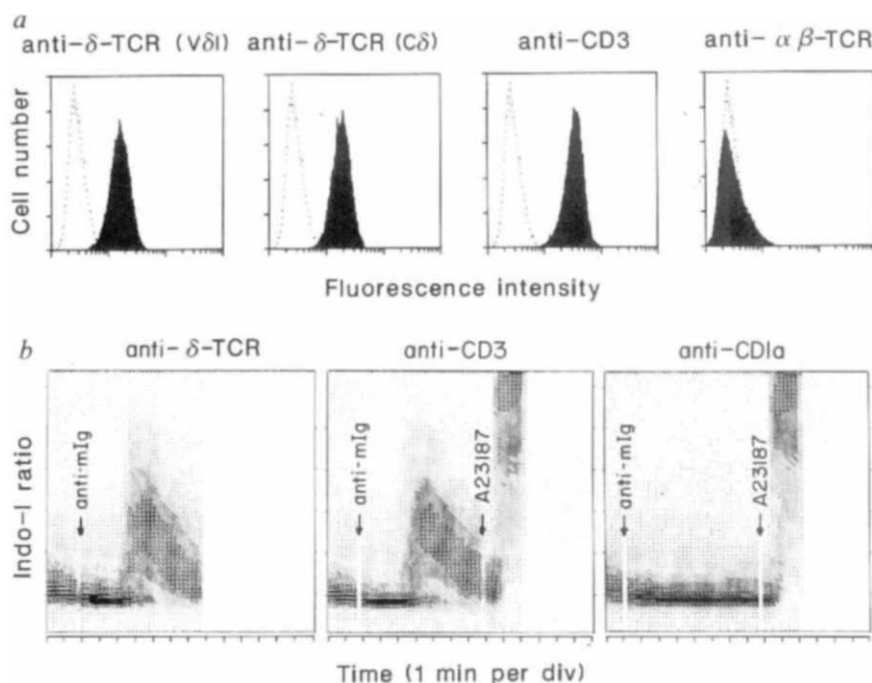
can substitute for α in pairing with β to form a $\beta\delta$ heterodimer. This receptor is expressed on the cell surface of the T-leukaemia cell line DND41 as analysed with β - and δ -specific monoclonal antibodies. We suggest that a variety of factors including, for example, the deletion of the δ -TCR loci, can now be understood as exclusion mechanisms operating to prevent not only the formation of $\gamma\delta$ receptors, but also of $\beta\delta$ T-cell receptors, thereby promoting the numerically dominant $\alpha\beta$ TCR lineage. Nevertheless, some developing T cells that do not rearrange the α -loci may express the $\beta\delta$ TCR as described here.

Cell-surface staining of the thymus-derived human T-leukaemia cell line DND41 (ref. 14) showed reaction with monoclonal antibodies δ TCS1 and anti-TCR δ 1, which are specific for δ -TCR variable and constant epitopes respectively^{15,16} (Fig. 1a; H. Band, personal communication). Staining was also observed with antibodies against CD3 and CD4. DND41 cells did not react with antibodies BMA031 or WT31 which are specific for the $\alpha\beta$ TCR complex (Fig. 1a)^{17,18}.

Immunoprecipitation from ¹²⁵I-labelled DND41 cells was used to characterize the CD3-associated TCR chains. Using either anti-CD3 or anti- δ -TCR monoclonal antibodies, we

FIG. 1 a, Cell-surface phenotype of DND41 cells. Cytofluorographs of DND41.C1 cells stained with IgG1 monoclonal antibodies δ TCS-1 (anti-V δ 1), anti-TCR δ 1 (anti-C δ), anti-Leu4 (anti-CD3) or IgG2b antibody BMA031 (anti- $\alpha\beta$ TCR) are shown in black in relation to staining with IgG1 antibody P3 (control), represented by the dotted line. b, Crosslinking of the $\beta\delta$ TCR-CD3 complex results in a [Ca²⁺]_i response. Indo-1 loaded DND41.C1 cells were labelled with either IgG1 antibody anti-TCR δ 1 (anti- δ -TCR), IgG2a antibody 64.1 (anti-CD3) or IgG1 antibody OKT6 (anti-CD1a) monoclonal antibodies as first antibody. Anti-mouse immunoglobulin-specific serum was added at the indicated time for crosslinking. The indo-1 ratio that represents the relative cytoplasmic free Ca²⁺ level was determined over time. Addition of calcium ionophore A23187 caused a maximal response.

METHODS. To obtain cells expressing high levels of CD3 and to assure clonality, the DND41 cell line was subcloned by limiting dilution and representative clones DND41.C1 and DND41.C2 were examined in detail. For cytofluorographic analysis, cells were incubated with a saturating amount of antibody ascites or purified antibody on ice for 45 min, washed, incubated with FITC-conjugated goat F(ab')₂ anti-mouse (H+L) and analysed on a FACScan flow cytometer (Becton-Dickinson). To measure calcium responses, cells were incubated in 5 μ M indo-1 acetoxymethyl ester³¹ (Molecular Probes) in 2% FCS/RPMI 1640, pH 7.2, at 10⁷ cells ml⁻¹ at 37 °C for 30 min, labelled with saturating amount of antibody ascites on ice for 25 min and washed in ice-cold 2% FCS/PBS containing 0.2 mM CaCl₂ and 5 mg ml⁻¹ glucose. Cells were analysed at 37 °C by excitation of indo-1 at 351–363 nm and registration of the emission at 400–410 nm (violet) and 480–490 nm (blue) on an Epics 750 flow



cytometer (Coulter Electronics). The depicted emission ratio between violet and blue (indo-1 ratio) is an indication of the relative cytoplasmic free [Ca²⁺]_i level. At the indicated time, 20 μ l cross-linking rabbit anti-mouse immunoglobulin serum was added to the cells. The maximum ratio was determined by addition of 10 μ g ml⁻¹ of A23187 ionophore (Calbiochem).

resolved radiolabelled cell-surface proteins of relative molecular masses (M_r) 43,000 (43K) and 37K by SDS-PAGE under reducing conditions, as well as the CD3 complex (Fig. 2a, lanes 6 and 8). The two TCR subunits were disulphide-linked because they migrated at 70K in SDS-PAGE under non-reducing conditions (Fig. 2a, lanes 5 and 7) and as off-diagonal species in two-dimensional SDS-PAGE (data not shown). The following experiments show that the heterodimer on DND41 cells corresponds to a $\beta\delta$ and not a $\gamma\delta$ TCR.

To identify the chain partner of the δ -TCR subunit in the heterodimer, we carried out immunoprecipitations with the well characterized monoclonal antibody β Framework1 (β F1), which is specific for a determinant present on all β -TCR subunits¹⁹. The antibody β F1 identified the 70K dimer recognized also by anti-TCR δ 1 (Fig. 2a, lanes 3 and 5). With both immunoprecipitates, the 70K dimer resolved into 43K and 37K species under reducing conditions (Fig. 2a, lanes 4 and 6). We confirmed that the 70K dimer contained both β - and δ -TCR subunits with another β -TCR specific antibody reagent, H38 (ref. 20) and anti-TCR δ 1, both of which recognize the heterodimer in western blots (Fig. 2c, lanes 2 and 3).

In support of our immunochemical results, the presence of full-length (as well as truncated) β -TCR and δ -TCR transcripts was demonstrated by northern blotting, using ³²P-labelled C β and C δ probes respectively (Fig. 3a, lanes 4 and 5). In fact, a V δ 1-specific complementary DNA probe hybridized with the full-length δ -TCR transcripts from DND41 cells (Fig. 3a), consistent with δ TCS1 antibody reactivity, which correlates with

V δ 1 usage. Southern-blot analyses with J β probes revealed one J β 1 and one J β 2 rearrangement, and hybridization with the J δ 1 probe showed the expected V δ 1-J δ 1 rearrangement (Fig. 3b, lanes 2)²¹. To establish the complete structure of the β - and δ -TCR proteins, we cloned and sequenced full-length, in-frame β - and δ -TCR cDNA clones from a DND41 λ gt10 library. These cDNA clones correspond to normal β -TCR (V β 18.1-D β 1.1-J β 1.2-C β 1) and δ -TCR (V δ 1-D δ 1-D δ 2-J δ 1-C δ) transcripts (Fig. 3c).

This apparent association of β - with δ -TCR led us to ask whether γ - or α -TCR products were present in these cells. Biosynthesis of γ -TCR protein was not detectable in DND41 cells by immunoprecipitation with the framework γ -TCR reagent, anti-C γ M1 (ref. 22), although abundant γ -TCR protein was synthesized under these same conditions in $\gamma\delta$ TCR-bearing cell lines such as PEER (Fig. 2d, lanes 2 and 3). DND41 cells do contain full-length γ -TCR transcripts (Fig. 3a, lanes 4 and 5). However, Southern-blot analyses using a γ -TCR constant-region probe revealed the deletion of the C γ 1 genes (Fig. 3b, lane 2). As the remaining C γ 2 genes only code for non-disulphide-linked TCRs (ref. 22), the disulphide-linked heterodimer on these cells could not be encoded by the C γ genes available. Given this, and the fact that we detected no biosynthesis of γ -TCR protein, we assume that these γ -TCR transcripts result from non-functional γ -TCR rearrangements. Indeed, sequence analysis of two distinct γ -TCR cDNA clones revealed that both transcripts were out-of-frame at the V-J junctional region (Fig. 3d).

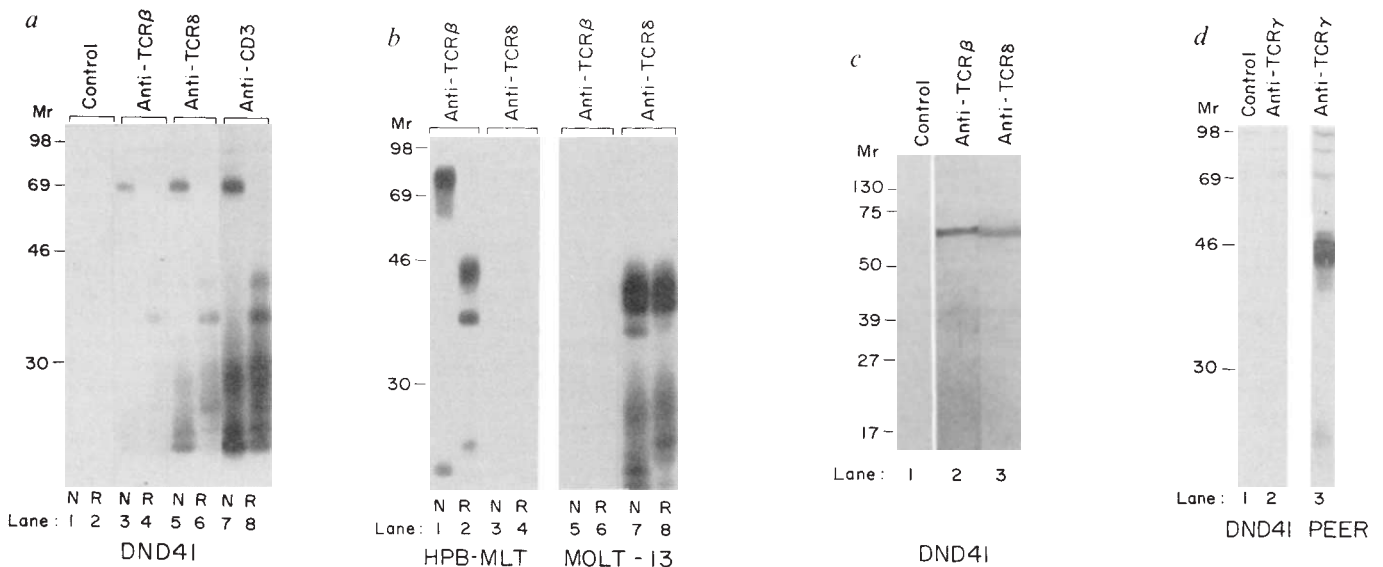
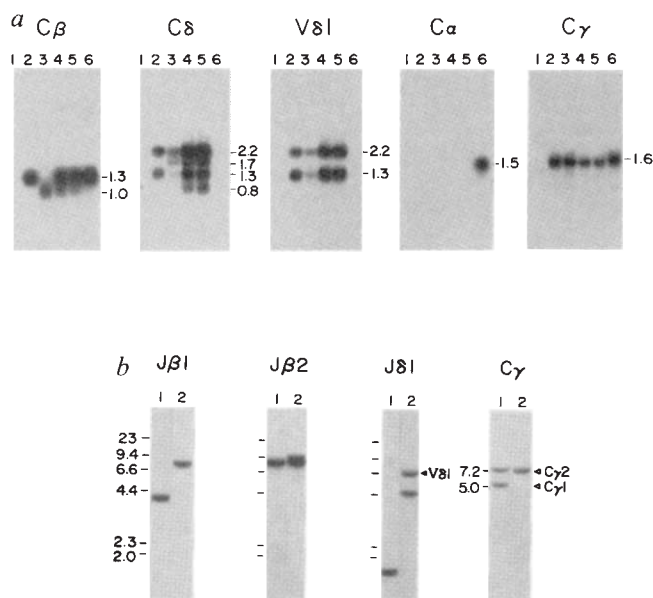


FIG. 2 *a*, Immunological analysis of the $\beta\delta$ TCR heterodimer. Lysates from cell surface ¹²⁵I-labelled DND41 cells were immunoprecipitated with IgG1 antibodies P3 (control), β F1 (anti- β -TCR), anti-TCR δ 1 (anti- δ -TCR) and anti-Leu4 (anti-CD3). Immunoprecipitates were resolved on a 10% SDS-polyacrylamide gel under non-reducing (N) or reducing (R) conditions. Note that both the anti- β -TCR and anti- δ -TCR-specific antibodies react with the heterodimer. $M_r(\times 10^{-3})$ markers are shown at the left of each panel. *b*, Immunological analysis of $\alpha\beta$ TCR and $\gamma\delta$ TCR heterodimers. Lysates from ¹²⁵I-labelled $\alpha\beta$ TCR-bearing HPB-MLT cells and $\gamma\delta$ TCR-bearing MOLT-13 cells were immunoprecipitated with β F1 (anti- β -TCR) and anti-TCR δ 1 (anti- δ -TCR). Immunoprecipitates were resolved on a 10% SDS-polyacrylamide gel under non-reducing (N) or reducing (R) conditions. Note that the anti- δ -TCR antibody does not react with the $\alpha\beta$ TCR heterodimer and that the anti- β -TCR antibody does not react with the $\gamma\delta$ TCR heterodimer. *c*, Western blot analysis of the $\beta\delta$ TCR heterodimer. Total membrane extracts of DND41 cells were resolved by 10% SDS-PAGE under non-reducing conditions, transferred to a nitrocellulose membrane and probed with normal rabbit serum (control), H38 serum (anti- β -TCR) or antibody anti-TCR δ 1 (anti- δ -TCR). *d*, DND41 cells do not synthesize detectable γ -TCR protein.

DND41 and PEER cells were biosynthetically labelled with [³⁵S]methionine and [³⁵S]cysteine for 5 h. Lysates of similar cell equivalents were immunoprecipitated with P3 (control) and anti-C γ M1 (anti- γ -TCR) and the products resolved by 10% SDS-PAGE under non-reducing conditions. METHODS. Cells were radio-iodinated using lactoperoxidase or biosynthetically labelled with [³⁵S]methionine and [³⁵S]cysteine, solubilized in 1% digitonin in Tris-buffered saline (TBS) solution containing 1 mM PMSF and 8 mM iodoacetamide, and immunoprecipitated as described²². For western blot analysis, DND41 cells were disrupted by nitrogen cavitation. A membrane fraction was prepared, solubilized in 1% Triton X-100/TBS containing PMSF and iodoacetamide, resolved at 6×10^7 cell equivalents per lane on a 10% SDS-polyacrylamide gel under non-reducing conditions, and transferred to a nitrocellulose membrane. Membrane strips were preincubated in 5% milk protein containing TBS, incubated overnight in a 1:1,000 dilution of control or H38 rabbit serum, or in a 1:500 dilution of anti-TCR δ 1 ascites in 10% FCS/TBS containing 0.02% sodium azide at 4 °C, then washed, incubated in alkaline phosphatase-conjugated anti-rabbit or anti-mouse IgG F(ab')₂ fragments, washed again and developed in nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate-containing detection buffer¹⁹.



C $V_{\beta 18.1}$ N $D_{\beta 1.1}$ N $J_{\beta 1.2}$
 F C A S S P G T G R Y G Y T F G
 ttctgtgccagctcacc c gggacaggg aga tatggctacaccttcggt

$V_{\delta 1}$ N $D_{\delta 1}$ N $D_{\delta 2}$ N $J_{\delta 1}$
 C A L G E A F R P S R T F T D K L I F
 tgtgtctcttggggaa g ccttcc gaccatc acg caccttc accgataaacatcattt

d $V_{\gamma 1.5}$ N $J_{\gamma 2.3}$
 V Y Y C A T G H L L L U
 gtctattactgtgccacc ggccacctt ttattataagaacctcttggcagtggaacaacactgtt
 Y Y K K L F G S G T T L V

$V_{\gamma 1.8}$ N $J_{\gamma 2.3}$
 V Y Y C A T W D T U
 gtctattactgtgccacctggga cacataaggggg acaacactgtt
 T T L V

FIG. 3 *a*, TCR gene expression in $\beta\delta$ TCR-, $\gamma\delta$ TCR- and $\alpha\beta$ TCR-bearing T-cell lines. Northern blots with total cellular RNA from the promyelomonocytic line HL60 (lane 1), the $\gamma\delta$ TCR lines PEER (lane 2) and MOLT-13 (lane 3), the $\gamma\delta$ TCR clone DND41.C1 (lane 4), DND41.C2 (lane 5) and $\alpha\beta$ TCR line HPB-ALL were hybridized with TCR constant- or variable-region probes, as indicated at the top. Note that DND41 contains full-length β -TCR and δ -TCR mRNA, but lacks α -TCR mRNA. Transcript sizes of α -, β -, γ -, and δ -TCR (indicated in kilobases) are based on published lengths. *b*, TCR gene rearrangements in DND41 cells. Genomic DNA from HL60 (germline control, lanes 1) and DND41 (lanes 2) was analysed by Southern blotting using the probes indicated at the top. Analysis using a γ -TCR constant-region probe revealed deletion of the $C\gamma 1$ gene segment on both chromosomes, indicating that both $V\gamma$ to $J\gamma$ rearrangements must splice to $C\gamma 2$ gene segments³². *Hind*III-digested DNA was hybridized with $J\beta 1$ or $J\beta 2$ probes, *Xba*I-digested DNA with a $J\delta 1$ probe and *Eco*RI-digested DNA with a $C\gamma$ probe. *c*, Partial nucleotide and deduced-amino-acid sequence of β -TCR and δ -TCR cDNA clones. Full-length clones β DND21 and δ DND1 from a DND41 cDNA library were sequenced and found to represent in-frame transcripts. The variable (V), diversity (D) and joining (J) region gene segments were identified by comparison with genomic and cDNA sequences^{33,34}. Nucleotides located between these gene segments are presumably template-independent additions denoted as N regions (N). The nucleotide sequences are depicted in small letters and the deduced amino-acid sequence in single-letter capitals (one-letter code). Northern blot analysis with a V_{β DND21 probe detected full-length mRNA from DND41.C1 and DND41.C2 cells (data not shown). *d*, Partial nucleotide and deduced-amino acid sequences of two γ -TCR cDNA clones. Nearly full-length clones γ DND1.5 and γ DND1.8 were derived from polymerase chain reaction amplified DND41 cDNA, sequenced and found to represent out-of-frame transcripts. Nucleotide sequences are

depicted in small letters with the deduced amino-acid sequences in single-letter capitals above. As a reference the in-frame deduced amino-acid sequences of the $J\gamma$ gene segments are depicted below the nucleotide sequences. The one-letter code U marks termination codons. Note that in the upper transcript sequences corresponding to $V\gamma 1.5$ and $J\gamma 2.3$ are joined imprecisely resulting in a translational frameshift, while in the lower transcript sequences corresponding to $V\gamma 1.8$ and $J\gamma 2.3$ contain an in-frame termination codon in the N-region.

METHODS. RNA was prepared for northern blots by LiCl/urea lysis and electrophoresed at 10 μ g per lane through 1.5% agarose gels containing 2.2 M formaldehyde (ref. 21). For Southern blots, genomic DNA was digested with restriction enzymes and electrophoresed at 5 μ g per lane through 0.7% agarose gels. After transfer, RNA and DNA were ultraviolet-crosslinked to Hybond-N membranes (Amersham) and hybridized with α -³²P-labelled probes. cDNA probes were: $C\alpha$ (a 389-bp *Pvu*II fragment of pGA5; ref. 35); $C\beta$ (a 392-bp *Bgl*II fragment of 12A1; ref. 36); $C\gamma$ (a 855-bp *Xmn*I fragment of IDP2.11; ref. 37); $C\delta$ (clone 0-240; ref. 13); $V\delta 1$ (a 315-bp *Eco*RI-*Scal* fragment of 0-240/47; ref. 13); genomic probes were: $J\beta 1$ (a 2.6-kb *Hind*III-*Nsi*I fragment; ref. 38); $J\beta 2$ (a 4.4-kb *Eco*RI fragment; ref. 38); and $J\delta 1$ (a 1.7-kb *Xba*I fragment; ref. 21). A λ gt10 library was constructed from 5 μ g of DND41 poly(A)⁺ RNA and screened with a $C\beta$ and $V\delta 1$ probe²². The cDNA clones β DND21 and δ DND1 were sequenced by the dideoxy chain termination method after subcloning into M13 vectors. To obtain the γ -TCR sequences shown in Fig. 3d we used the following strategy. Southern blot analyses using a $J\gamma 2.3$ genomic probe identified a $V\gamma 1.8$ - $J\gamma 2.3$ and a $V\gamma 1.5$ - $J\gamma 2.3$ rearrangement in *Eco*RI-digested DND41 DNA (data not shown). Therefore we amplified cDNA derived from DND41 poly(A)⁺ RNA using the polymerase chain reaction (PCR)³⁹ with a primer complementary to the 5' untranslated region of the $V\gamma 1$ -genes (5'-d[GGGGTCGACTTTAAGAGGATCTTCTGCTCC]) and a primer complementary to the third exon of the γ -TCR constant region (5'-d[GTGTTGTGAGCTGCAGCAGTAGTGTA]). The 890-basepair PCR product mainly consisted of $V\gamma 1.5$ -containing cDNA clones. To isolate $V\gamma 1.8$ -containing cDNA clones the PCR product was digested with the restriction enzyme *Dde*I that cuts $V\gamma 1.5$ -containing clones but not $V\gamma 1.8$ -containing clones. The *Dde*I-resistant PCR product was reamplified using PCR.

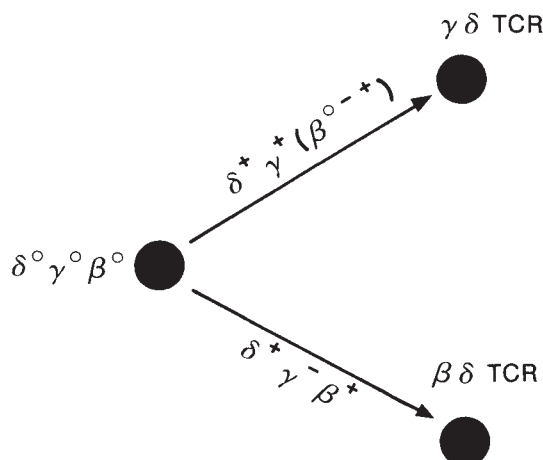


FIG. 4 Rearrangement requirements for expression of δ -containing T-cell receptors. As γ - and δ -TCR, like α - and β -TCR, form preferred chain pairs, each subunit will join with its partner, if available. Thus, $\gamma\delta$ TCR is the expressed heterodimer when both of these chains are functional, irrespective of what state β -TCR assumes. But we propose that if γ -TCR is non-functional in a cell containing functional β - and δ -TCR, then these subunits will pair and reach the cell surface as a $\beta\delta$ T-cell antigen receptor. In these cells, the α -TCR loci must be in germline configuration. Minus sign, non-functional; plus sign, functional; O, not rearranged.

In addition, we find that DND41 cells do not transcribe α -TCR, because a ^{32}P -labelled α probe failed to detect messenger RNA transcripts in northern blots (Fig. 3a, lanes 4 and 5). Indeed, the $J\alpha$ region is in germline configuration on both chromosomes in this cell line^{23,24}. Karyotype analysis of DND41 cells revealed no evidence for translocations or inversions involving either chromosome 7 (containing the β - and γ -TCR loci) or chromosome 14 (containing the δ - and α -TCR loci), indicating that hybrid receptor chains should not occur in these cells (data not shown). Taken together, these results indicate that the heterodimer on DND41 cells is composed of normal β - and δ -TCR subunits and does not include either γ -TCR or α -TCR chains.

We then determined whether the $\beta\delta$ TCR-CD3 complex could be functional and transduce an activation signal to the cell. Indo-1-loaded DND41 cells were labelled with anti-TCR δ 1 or anti-CD3 antibodies and their TCRs crosslinked using mouse immunoglobulin-specific antiserum while continuously monitoring the cytoplasmic free Ca^{2+} concentration. Both antibodies induce a transient increase in the internal concentration $[\text{Ca}^{2+}]_i$ in the presence of crosslinking antiserum (Fig. 1b). There was no such change in $[\text{Ca}^{2+}]_i$ after crosslinking of CD1a molecules or transferrin receptors (Fig. 1b, and data not shown).

The association of β -TCR with δ -TCR we describe here was unexpected, because to date only α -TCR has been found with β , and γ -TCR with δ on T-cell surfaces^{25,26}. In fact, in PEER cells it has been shown that when β -, γ - and δ -TCR are synthesized in the same cell, only the $\gamma\delta$ TCR pair is expressed on the cell surface^{5,27}. We assume that β -TCR is able to physically pair with δ because of certain structural features shared by δ -TCR and α -TCR, such as their constant domain sizes and their spacing of basic residues in the proposed transmembrane region¹³. However, the pairing of β - with δ -TCR (rather than with α) may occur only in the absence of their preferred partners. There is a similarly preferred association for isotypically matched major histocompatibility complex class II A α A β and E α E β pairs, isotype-mixed A β E α dimers probably occurring only when the preferred partners are unavailable²⁸. During T-cell development δ -, γ - and β -TCR may be expressed at the same time, before α -TCR rearrangement²⁶. It is possible that developing thymocytes which fail to productively rearrange their γ -TCR may synthesize β - and δ -TCR subunits and express these stably on the cell surface, as happens on DND41 cells (Fig. 4).

The issue of whether $\gamma\delta$ -TCR and $\alpha\beta$ -TCR result from sequential rearrangements of the TCR loci²⁹ or as separate lineages^{12,30} is controversial. The potential occurrence of a $\beta\delta$ -TCR provides a further level of complexity in these T-cell lineage relationships. Nevertheless, as a δ -TCR containing heterodimer, the events which operate to separate δ -TCR and α -TCR expression are likely to apply similarly to $\gamma\delta$ - and $\beta\delta$ -bearing cells, both of which require the α -locus to be in germline configuration and the δ -locus to be rearranged. The ability of δ -TCR and α -TCR to pair with β -TCR seems to be managed during development. Mechanisms controlling gene expression, the organization of the α/δ -TCR locus, and physical properties that govern chain pairing together are crucial features in regulating the frequency of expression of $\alpha\beta$ TCR, $\gamma\delta$ TCR and potentially $\beta\delta$ TCR. Further studies will be necessary to determine the occurrence and function of cells expressing the $\beta\delta$ TCR.

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Binding of myosin I to membrane lipids

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THE single-headed myosins called myosin-I were first isolated from the protozoan *Acanthamoeba*¹ and subsequently identified in other cells (reviewed in ref. 2). We previously reported evidence that myosin-I is responsible for the movement of membranes, extracted from *Acanthamoeba*, along actin filaments *in vitro*³. Here we show for the first time that myosin-I can bind directly to NaOH-extracted membranes isolated from *Acanthamoeba* and to vesicles of pure lipids with an affinity sufficient for extensive binding in the cell. Membrane-bound myosin-I may provide a mechanism for many cellular movements previously thought to involve filamentous myosin-II.

Although myosin-I was originally isolated as a soluble enzyme, four lines of evidence suggest a membrane association. First, fluorescent-antibody localization⁴⁻⁶ showed *Acanthamoeba* myosin-I concentrated near the plasma membrane. Second, the radial spoke between the actin filament core and the surrounding plasma membrane in intestinal microvilli⁷ has been identified as myosin-I (see ref. 8 for a review). Third, membranous vesicles isolated from *Acanthamoeba* can move along actin filaments via an attached motor tentatively identified as myosin-I by antibody inhibition³. Fourth, substantial amounts of *Acanthamoeba* myosin-I are associated with membranes fractionated by density-gradient centrifugation³.

To look directly for myosin-I binding to membranes we have performed reconstitution assays using purified myosin-IA and -IB from *Acanthamoeba* and NaOH-extracted membranes^{9,10} from *Acanthamoeba* organelles. Extraction of peripheral membranes proteins, particularly actin¹¹⁻¹³ and myosin-I itself, from the membranes was considered a necessary first step to the demonstration of a direct binding site for myosin-I.

In a buffer containing physiological concentrations of ATP and salt, purified myosin-IA and -IB bind to membranes

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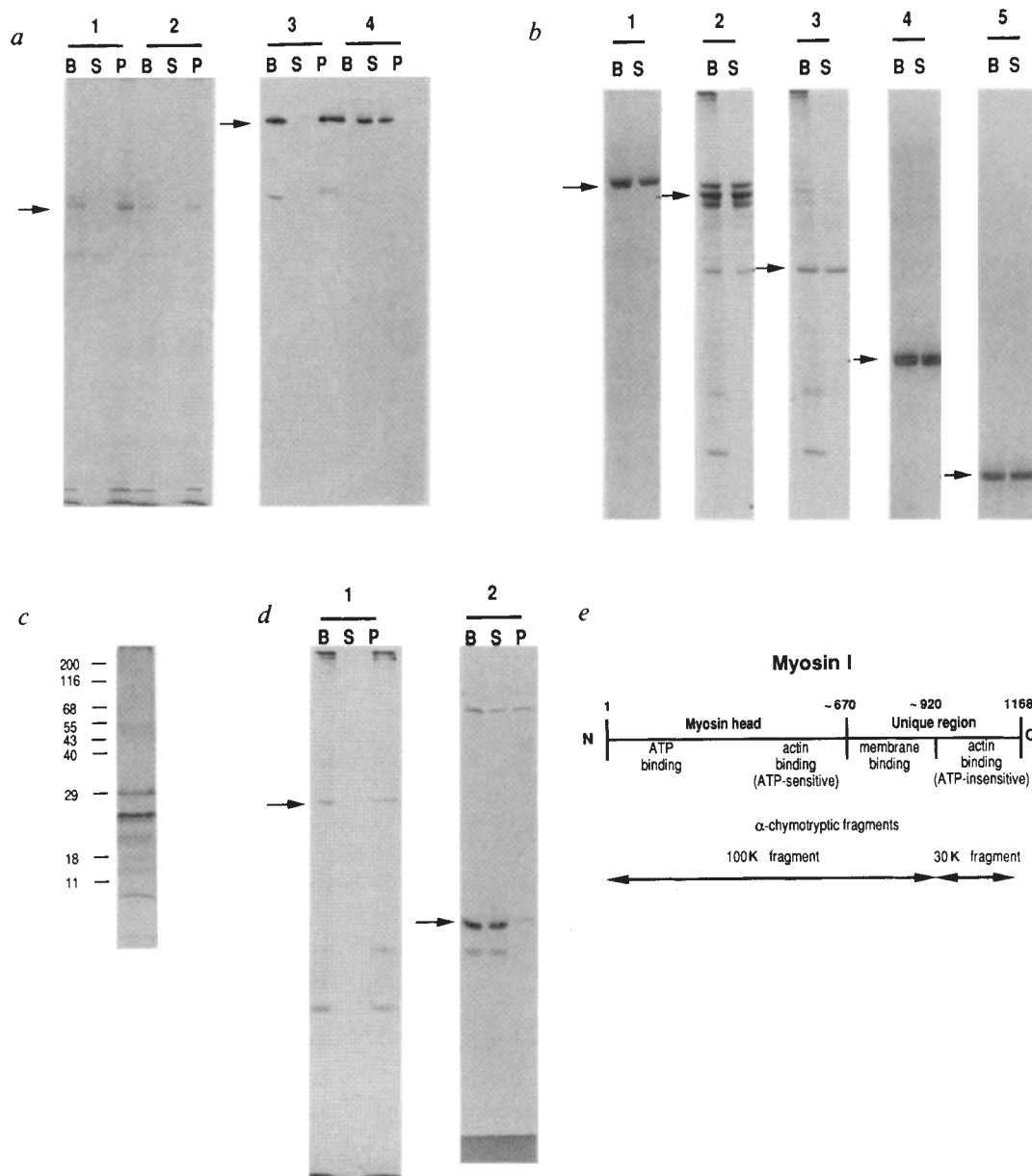


FIG. 1 *Acanthamoeba* myosins-IA and -IB bind to extracted membranes, whereas other purified proteins do not. Bound myosin-I was separated from free myosin-I by centrifugation. B, samples before centrifugation; S, supernatant after centrifugation; P, pellet. These fractions were analysed by SDS polyacrylamide gel electrophoresis²³. A membrane concentration equivalent to ~100 μ g of membrane protein was used in these experiments. a, Binding to membranes. The gels in 1 and 2 were stained with coomassie blue. 1, myosin-IA pellets with membranes; 2, myosin-IB pellets with membranes. Gels in 3 and 4 were immunoblots prepared by transfer onto nitrocellulose, stained with a monoclonal antibody to myosin-I (ref. 5) and visualized with peroxidase-conjugated goat anti-mouse immunoglobulin. 3, myosin-IB pellets with membranes 4, myosin-IB alone remains in the supernatant. b, Reaction of other purified proteins with extracted membranes. Gels stained with coomassie blue. 1, *Acanthamoeba* myosin II; 2, rabbit skeletal muscle heavy meromyosin; 3, rabbit skeletal muscle myosin subfragment 1; 4, bovine serum albumin and 5, ovalbumin. c, Proteins of NaOH-extracted membranes stained with silver. The 100 K chymotryptic fragment pellets with the membranes. The 30 K fragment is not visible on this 8% gel. d, Reaction of chymotryptic fragments of myosin-IA (ref. 14) with extracted membranes. 1, Coomassie blue stain. The 100 K chymotryptic fragment pellets with the membranes. The 30 K fragment is not visible on this 8% gel. 2, An immunoblot of a 12.5% gel shows that the 30 K fragment remains in the supernatant (S) when the membranes pellet. e, Schematic of myosin-I heavy chain based on the nucleotide sequence published for *Acanthamoeba* myosin-IB (ref. 15) and proteolytic fragments¹⁴ of myosin-IA.

METHODS. The use of the method described in ref. 14 routinely yielded a

total 20–30 mg of the two similar isozymes named myosins-IA plus -IB from 1 kg of *Acanthamoeba*. Rabbit skeletal muscle myosin was prepared by the method of Kielley and Harrington²⁴. Ovalbumin, bovine serum albumin (BSA), were purchased from Sigma. Heavy meromyosin and subfragment-1 of rabbit skeletal muscle myosin were prepared by the methods of Margossian and Lowey²⁵. Chymotryptic fragments of myosin-IA were prepared by the method described in ref. 14. Membranous organelles were prepared at 0–4 °C from ~20 g of *Acanthamoeba*. Cells were suspended in 2 volumes of extraction buffer (60 mM potassium glutamate, 10 mM imidazole-HCl, pH 6.4, 2 mM EGTA, 2 mM MgCl₂) supplemented with 4 mM ATP, 1 mM PMSF (phenylmethylsulphonyl fluoride) and 18 μ g ml⁻¹ benzamidine, broken with 20 strokes of a tight-fitting Dounce homogenizer and centrifuged for 3 min at 3,000 r.p.m. in a Beckman JA 20 rotor. The supernatant was further centrifuged for 90 min at 38,000 r.p.m. in a Beckman Ti 45 rotor. The brown membrane pellet was resuspended in homogenization buffer with 1 mM ATP; care was taken only to resuspend the membrane pellet, not the clear poly-ribosome pellet. Extracted membranes were made using the method of Luna⁹, as follows. Membranes were washed by dilution to 50 ml in extraction buffer with 1 mM ATP and centrifuged for 60 minutes as above. The membrane pellet was resuspended in 50 ml of 0.1 M NaOH with 1 mM dithiothreitol homogenized and then sonicated for 30 s. This extract was centrifuged as above for 30 min. The pellet was homogenized in 50 ml extraction buffer and recentrifuged for 30 min. The pellet was finally homogenized in 10 ml extraction buffer and frozen in liquid nitrogen and stored at -80 °C for several weeks. Pelleting assays were performed as described in Fig. 2.

(Fig. 1a) stripped of all actin, myosin-I and other peripheral proteins with 100 mM NaOH (Fig. 1c). No myosin-I pellets in the absence of membranes, and none of various other purified proteins, including other myosins isozymes, pellets with stripped membranes under these conditions (Fig. 1b). Myosin-I still binds to stripped membranes after they are digested extensively with α -chymotrypsin (data not shown). Binding of both myosin-IA and -IB to extracted membranes is inhibited by ~50% by the addition of 100 mM NaCl and by almost 100% by 250 mM NaCl.

The concentration dependence of the binding of purified myosin-I to stripped membranes is consistent with a dissociation constant of 140 nM and saturates at ~0.2–1.0 mg myosin-I per mg of membrane protein (Fig. 2). Given the heterogeneity of the membrane polypeptides (Fig. 1c), the binding capacity of the membranes for myosin-I far exceeds the molar concentration of any single membrane protein.

Experiments with proteolytic fragments of myosin-I (ref. 14) tentatively localize the membrane binding site to a part of the heavy chain between residues 670 and 920 (Fig. 1d, e). First, a 30 K (relative molecular mass, M_r) C-terminal fragment containing the ATP-insensitive binding site¹⁴ does not bind to the stripped membranes (Fig. 1d). Second, a 100 K N-terminal fragment, consisting of a typical myosin-head sequence up to residue 670 (ref. 15), binds to membranes (Fig. 1d). Because neither *Acanthamoeba* myosin-II nor head fragments of muscle myosin bind to membranes and because the head domain of myosin-I is unlikely to bind to any undetected actin in the membrane fraction under the conditions tested, the region of previously unknown function distal to residue 670 is probably

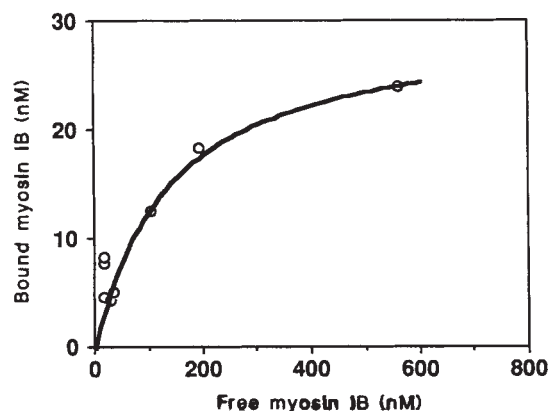


FIG. 2 Concentration dependence of myosin-IB binding to NaOH-extracted membranes. The solid line is a theoretical plot for $K_d = 139$ nM. A second experiment gave $K_d = 137$ nM. The binding capacity was 0.2–1.0 mg myosin-1 mg^{-1} membrane protein, depending on the protein assay used, because the Bradford²⁶ and Lowry²⁷ assays (with or without (SDS) give different estimates of membrane protein.

METHODS. Various concentrations of myosin-I were mixed with membranes containing ~5 μg of protein 0.2 ml extraction buffer containing, 1 mM ATP and 1 mg ml^{-1} (BSA) and pelleted as described below. The samples were run on a gel with a range of myosin-IB internal standards. Proteins were transferred onto nitrocellulose then reacted with an iodinated²⁸ monoclonal antibody (M1.8) against myosin-I (ref. 5). An autoradiogram of the blot was used as a guide to cut out individual myosin-I bands which were then counted in a γ -counter. The standard curve obtained from the internal standards was fitted with a polynomial curve and the experimental values interpolated from this curve. Pelletting assays were performed in a Beckman TL100 centrifuge. Myosin-I was mixed with organelles in a total volume of 0.2 or 0.4 ml in homogenization buffer plus 1 mM ATP. In all quantitative experiments this buffer contained 1 mg ml^{-1} BSA to minimize nonspecific interactions with the walls of the tube. Samples (20 μl) were taken of the starting mixture before centrifugation. In a number of experiments a cushion of 15% sucrose in homogenization buffer was added below the sample; this allowed cleaner recovery of the pellets. The tubes were centrifuged at 30,000 r.p.m. for 10 min at 20 °C. The supernatant was then removed. The pellet was resuspended in SDS sample buffer²³. Binding was followed by western blots of samples electrophoresed in SDS on 8% polyacrylamide gels²³.

responsible for binding. We do not exclude acylation of this region.

Myosin-I binds to purified membranes in a gel-filtration assay (Fig. 3). Free myosin-I elutes near the salt volume and is well separated from myosin-I associated with vesicles in the void volume (Fig. 3, band 2). Similar results are obtained with vesicles of *Acanthamoeba* lipids, pure phosphatidyl serine or pure phosphatidyl inositol 4,5-bisphosphate $\text{PtdIns}(4,5)\text{P}_2$ (Fig. 3, band 3–5). Myosin-I does not bind to vesicles of pure phosphatidyl choline under these conditions (Fig. 3 band 6). These results suggest that myosin-I binds to negatively charged surfaces provided by anionic phospholipids.

Reconstruction of the binding of myosin-I to membranes stripped of peripheral proteins occurs under the same physiological conditions where a motile myosin-I-membrane complex was originally isolated from the cell³. The dissociation constant of the complex is consistent with extensive binding of myosin-I to membranes within the cell. Although only lipids are required to form this complex *in vitro*, an interaction with lipids is unlikely to impart much specificity. It seems likely that intrinsic or peripheral proteins, including actin, limit the lateral mobility of myosin-I in the plane of the membrane and lead to local differences in concentration¹⁶. The ATP-insensitive, actin-binding site near the C terminus of myosin-I is a particularly attractive candidate for such an anchor because the affinity ($K_d = 0.12$ – 0.34 μM) is sufficient for binding to actin, but weak enough

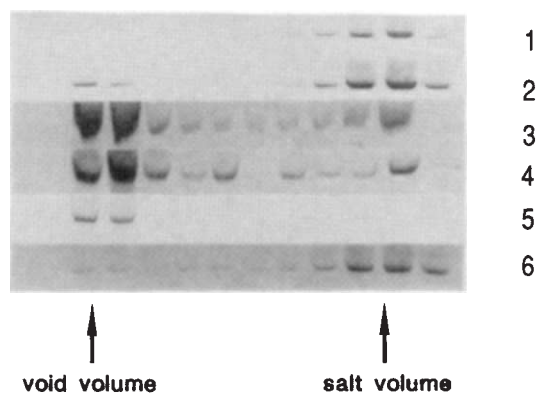
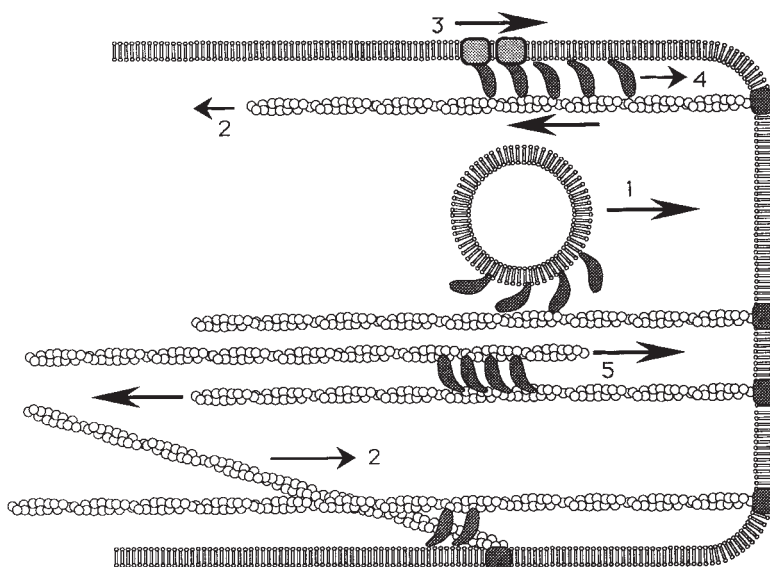


FIG. 3 Gel-filtration assay for myosin-I binding to membranes and lipid vesicles. A 0.7×19.0 cm column of Sepharose-cl 4B was run in homogenization buffer with 1 mM ATP and 1 mg ml^{-1} BSA. A 0.5-ml sample was loaded onto the column followed by 1 ml column buffer. The sample was then followed directly with the same buffer plus 0.3 M NaCl. This step was necessary because free myosin-I tended to stick to the column matrix² when not bound to other surfaces. Membrane-bound myosin-I passed unhindered through the column, ahead of the salt wash. Fractions of 0.6 ml were collected and the myosin-I visualized by western blotting from 8% gels. The column was loaded with myosin-IB mixed with (1) buffer alone; (2) NaOH-extracted membranes; (3) vesicles of *Acanthamoeba* lipids; (4) vesicles of pure phosphatidyl serine; (5) vesicles of pure $\text{PtdIns}(4,5)\text{P}_2$; and (6) vesicles of pure phosphatidyl choline.

METHODS. Lipids were prepared from *Acanthamoeba* by organic extraction²⁹. Briefly, 4.8 g of cells (washed three times) in 30 ml of 50 mM NaCl were extracted for 30 min after vortexing in 37.5 ml chloroform and 75 ml methanol. To this mixture was added 37.5 ml of H_2O plus 37.5 ml chloroform and the resulting suspension centrifuged to separate the organic and aqueous phases. The upper, aqueous phase was aspirated off to allow the lower, organic phase containing the extracted lipids to be dried under a steady stream of nitrogen gas. The lipid residue left was resuspended in chloroform and stored at -20 °C. To prepare these lipids for use in binding experiments, the solvent was again evaporated off with a stream of nitrogen gas and the residue resuspended in water by sonication. Protein was not detectable in this extract by a Bradford protein assay²⁶ or SDS-PAGE²³. Purified lipids (Sigma) were prepared for use by evaporating off the organic solvent used for storage and resuspended in water with extensive sonication at a concentration of 1 mg ml^{-1} . In the case of $\text{PtdIns}(4,5)\text{P}_2$ the lipid was added to homogenization buffer (containing divalent cations) used for the binding experiments at least 30 min before use. This permitted the conversion of the lipid from micelles to vesicles favoured under these conditions.

FIG. 4 Schemes for myosin-I-membrane interactions within the cell. 1, Membrane-bound myosin-I moves organelles along actin filaments within the cytoplasm; 2, myosin-I tethered in the membrane by association with other membrane proteins or a second actin filament produces a contraction on actin filaments. This could cause contraction of actin filaments tethered to cell structures such as the plasma membrane^{30,31}, or concentrate actin filaments into an area of activity; 3, myosin-I causes the movement of the plasma membrane relative to actin filaments in the cytoplasm. The direction of movement would be determined by the polarity of the actin filaments. At the cell periphery, in microvilli or filopodia the direction of this movement would be expected to be centrifugal. 4, Myosin-I and associated membrane molecules may be free to diffuse in the plane of the membrane. An interaction of myosin-I with tethered cortical actin filaments may serve to move myosin-I along the actin filaments, concentrating them in a region of activity. 5, Myosin-I has already been shown to cause contraction of actin filaments relative to each other, *in vitro*^{14,21,22}. A network of actin filament in the cytoplasm could be contracted in this way.



to allow dynamic rearrangements. Furthermore, it could be that if PtdIns(4,5)P₂ contributes to the binding site in the cell, phosphoinositide metabolism modulates the amount of myosin-I bound to membranes.

The continuation of many movements in cells depleted of myosin-II by genetic manipulations^{17,18} or by microinjection of inhibitory antibodies^{19,20} suggests that myosin-I powers various movements. *In vitro* studies^{14,21,22} show that myosin-I can cross-link and contract networks of actin filaments (Fig. 4, number 5). In addition, the association of myosin-I with membrane lipids provides potential mechanisms for at least four other types of movement (Fig. 4): (1) organelles along actin filaments in the cytoplasm; (2) membranes relative to actin filaments in the cytoplasm; (3) the plasma membrane relative to a tethered actin filament gel; and (4) membrane molecules relative to cortical actin filaments.

Coincident with the completion of this work, a paper from Miyata *et al.*³² has replicated some of the results described here and shows that myosin-I binds to purified plasma membranes.

Leucine zippers of *fos*, *jun* and GCN4 dictate dimerization specificity and thereby control DNA binding

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THE products of the *fos* and *jun* protooncogenes form a stable heterodimer which binds to the TPA-responsive element (TRE) TGACTCA with high affinity¹⁻⁹. These two proteins, together with the yeast GCN4 protein, belong to a growing family of transcription factors, including FosB, Fra1, JunB and JunD, whose members share a highly conserved DNA-binding domain¹⁰⁻¹⁴. This domain is composed of two structures: a basic motif, which is thought to bind directly to DNA; and a leucine zipper¹⁵, which provides a dimerization interface. Although this domain is highly conserved in Fos, Jun and GCN4, each of these three proteins has very different relative affinities for the TRE. To understand these differences, we used 'domain-swapping' experiments designed to test the relative contributions of the basic motif and the leucine zipper to TRE-binding affinity. Here we show that *fos*, *jun* and GCN4 have different affinities for the TRE due to differences in the hetero- or homo-dimerization capacity of their leucine zipper domains; the basic motifs of these three proteins have comparable DNA binding potential. These results indicate that leucine zippers control the types of protein complexes which can associate with a TRE and regulate gene expression.

We have previously shown¹ that the 92-residue Fos-core peptide (Fig. 1), which includes the Fos basic motif and the Fos leucine zipper, like Fos, cannot bind to the TRE alone, but can do so when in a dimeric complex with Jun. The lack of intrinsic DNA-binding activity of the Fos-core peptide may result from inability to form dimers⁴⁻⁷, or from the inability of the Fos basic motif in a Fos/Fos homodimer to interact with the TRE. To distinguish between these alternatives, we first asked whether the Fos-core peptide can dimerize with the full-length Fos

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FIG. 1 Structure and properties of chimaeric DNA binding domains. *a*, Diagrammatic representation of the Fos and GCN4 proteins. The positions of the Fos-core and GCN4-C83 peptides described below are indicated. F_B and G_B , basic motifs of Fos and GCN4, respectively; F_Z and G_Z , leucine-zipper domains of Fos and GCN4, respectively. *b*, The Fos-core peptide has a methionine instead of the codon at position 126 and a stop codon after the Fos residue 217. The construction of the pSP65 vector designed to express this peptide is described elsewhere¹. The GCN4-C83 peptide has eight heterologous amino acids at its N-terminus, the first of which is a methionine, and it terminates at the natural GCN4 stop codon¹⁶. A *Xho*I site was introduced between the basic motif and the leucine zipper of Fos-core and GCN4-C83. The introduction of this site generated two amino-acid substitutions in each peptide: residues 162 and 163 of Fos-core (T, D) and residues 250 and 251 of GCN4-C83 (M, K) were replaced by L, E. Using the introduced *Xho*I site, the basic motif of Fos-core was replaced with the basic motif of GCN4-C83 to generate the chimaeric peptide $G_B F_Z$ -core, and the leucine zipper of Fos-core was replaced with the leucine zipper of GCN4-C83 to generate the $F_B G_Z$ -core chimaera. To the right of the $G_B F_Z$ -core and $F_B G_Z$ -core chimaeras is a diagrammatic representation of the results discussed in the text.

METHODS. The *Xho*I site was introduced in GCN4-C83¹⁶ and Fos-core¹ by *in vitro* mutagenesis as described¹⁹. This *Xho*I site was used to clone the basic region of Fos-core and the leucine zipper of GCN4-C83 into pGEM-3 to generate pSP6 $F_B G_Z$ -core. The basic region of GCN4-C83 and the leucine zipper of Fos-core were cloned into pGEM-3 to generate pSP6 $G_B F_Z$ -core.

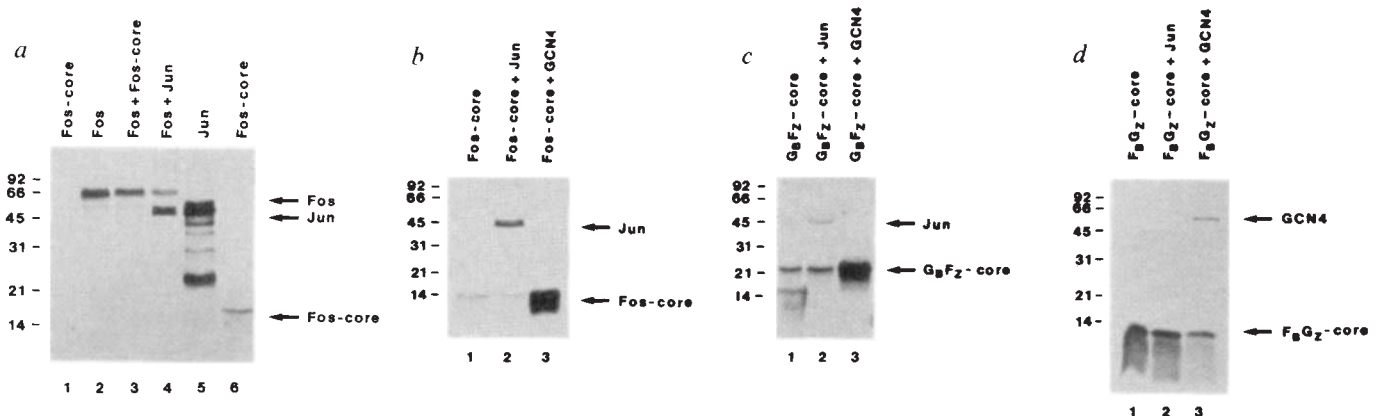
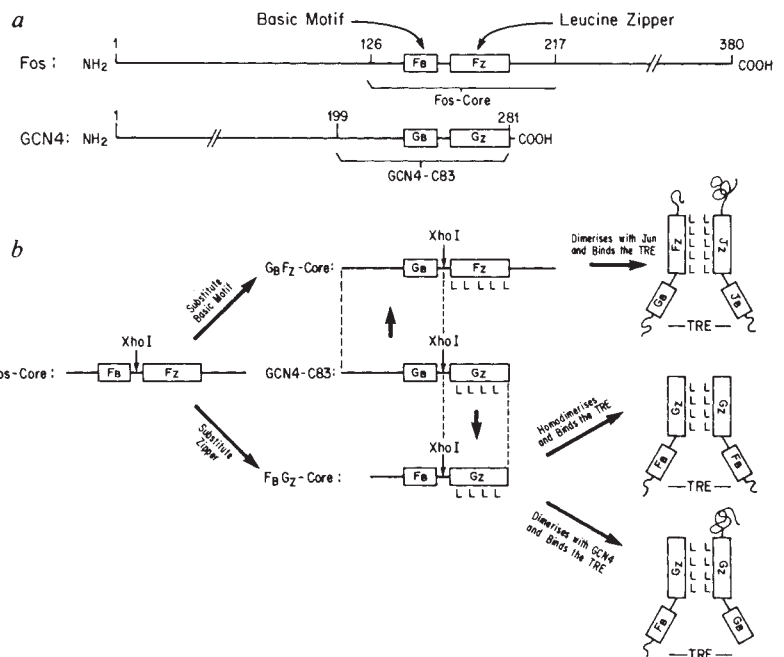


FIG. 2 *a*, A rabbit antiserum directed against the C-terminal residues of human c-Fos in frame with bovine growth hormone (from D. Slamon) was used to immunoprecipitate ³⁵S-labelled *in vitro* translation products of Fos-core (lane 1) or Fos (lane 2). The same antibody was used to precipitate the Fos protein after co-translation with the Fos-core peptide (lane 3) or after incubation with the Jun protein for 30 min at 37 °C (lane 4). The ³⁵S-labelled translation product of Jun is shown in lane 5 and the *in vitro* translation product of Fos-core precipitated with an antibody directed against TrpE-Fos fusion-containing residues 73-152 of the Fos protein²⁰ is shown in lane 6. *b*, The ³⁵S-labelled *in vitro* translation product of Fos-core was immunoprecipitated with a Fos antibody²⁰ either prior to (lane 1) or after a 37 °C, 30-min incubation with *in vitro* translated Jun (lane 2) or GCN4 (lane 3). *c*, A Fos antibody was used to immunoprecipitate *in vitro* translated $G_B F_Z$ -core (lane 1) or a co-translation of $G_B F_Z$ -core with either Jun (lane 2) or GCN4 (lane 3). *d*, A Fos antibody was used to immunoprecipitate the $F_B G_Z$ -core translation product either before (lane 1) or after a 37 °C, 30-min incubation of $F_B G_Z$ -core with Jun (lane 2) or GCN4 (lane 3). *e*, DNA binding activity of the *in vitro* translated chimaeric peptides analysed by the mobility shift assay. The *in vitro* synthesized products of GCN4, Jun, Fos-core, $F_B G_Z$ -core, $G_B F_Z$ -core (lanes 1-5) or a combination of these proteins (lanes 6-9) were incubated with a ³²P-labelled oligonucleotide of the 'fat-specific element' (FSE) (ref. 21) which contains a TRE.

METHODS. DNA fragments encoding the Fos-core, $G_B F_Z$ -core and $F_B G_Z$ -core products (see Fig. 1) under the control of the SP6 promoter as well as c-Fos²² and c-Jun⁷ cDNAs under the control of the T7 and T3 promoter, respectively, were transcribed *in vitro*²³ and then translated in a rabbit reticulocyte lysate, as described by the manufacturer (Promega). The immunoprecipitations were carried out as previously described¹ and the products were resolved by 15% (Fig. 2a) or 17% (Fig. 2b-d) SDS-PAGE. The markers on the left are in relative molecular mass (M_r) $\times 10^3$. For the

mobility-shift assay the 27-base pair FSE oligonucleotide (GATCCATGACTCA-GAGGAAACATACG) was end-labelled with [³²P] ATP using T₄ polynucleotide kinase and incubated at 37 °C for 30 min in the presence of various *in vitro* translated products as described previously¹. The proteins in lanes 6 and 9 were separately translated and then mixed with DNA whereas those in lanes 7 and 8 were co-translated before mixing with DNA. The products were resolved in a 4% non-denaturing polyacrylamide gel.

protein (Fig. 2a). We allowed Fos-core to form a complex with Fos and then asked whether an antibody that specifically recognizes the Fos protein (Fig. 2a, lane 2), but not Fos-core (lane 1) would indirectly immunoprecipitate the Fos-core product. Figure 2a, lane 3 shows that only the Fos protein is precipitated (compare with lane 6), indicating that Fos cannot dimerize with Fos-core, although in a parallel experiment it can dimerize with Jun (compare lanes 4, 5). These results support the idea that Fos and Fos-core are unable to bind the TRE alone because they cannot form homodimers.

The yeast transcription factor GCN4 has a DNA-binding domain very similar to that of the Fos/Jun family¹⁴ and contains a basic motif (G_B) adjacent to a leucine zipper (G_Z). The GCN4 DNA-binding domain, unlike that of Fos, can both homodimerize and bind the TRE with high affinity^{16,17}. Furthermore, peptides of the GCN4 leucine zipper form dimers¹⁸. We used domain-swapping to determine the relative contribution of the zipper and basic motif to the distinctive properties of GCN4 and Fos. We made the following two chimaeric peptides: (1) $F_B G_Z$ -core, a 68-residue peptide containing the basic motif of Fos (F_B) adjacent to the leucine zipper of GCN4 (G_Z); and (2) $G_B F_Z$ -core, a 107-residue peptide containing the basic motif of GCN4 (G_B) adjacent to the leucine zipper of Fos (F_Z) (Fig. 1b). Both peptides were generated using *in vitro* transcription followed by translation in a rabbit reticulocyte lysate.

We first asked whether the leucine zipper portion of these chimaeric peptides displayed the dimerization specificity of the parent peptide. Both chimaeric peptides are recognized by an antibody raised against a TrpE-Fos fusion protein. Each peptide was allowed to form a complex with either Jun or GCN4 and then immunoprecipitated with the anti-TrpE-Fos antibody to assay for complex formation. Figure 2d shows that the $F_B G_Z$ -core peptide, which carries the GCN4 leucine zipper, can form a complex with GCN4 but not Jun, and Fig. 2c shows that the $G_B F_Z$ -core peptide which carries the Fos leucine zipper can form a complex with Jun but not GCN4. This is a true reflection of the properties of the parental proteins as the Fos-core protein can form a complex with Jun but not GCN4 (Fig. 2a), whereas the GCN4 protein can form homodimers¹⁷ but cannot form a complex with Jun (data not shown).

We then tested the chimaeric peptides for their ability to bind an oligonucleotide containing a TRE (Fig. 2e). In this assay, the GCN4 DNA-binding domain binds a TRE with high affinity (lane 1); the Jun protein (lane 2) and the Fos-core peptide (lane 3) do not bind when assayed separately, but will bind with high affinity if added together (lane 6). When compared with these proteins, the $G_B F_Z$ -core chimaera did not bind to the TRE (lane 5) whereas $F_B G_Z$ -core bound with high affinity (lane 4). The ability of the $F_B G_Z$ -core peptide to bind the palindromic TRE in the absence of the Jun protein indicates that the basic motif of Fos is capable of direct DNA binding when incorporated into a homodimer through GCN4 zipper interaction (Fig. 1b). To confirm that $F_B G_Z$ -core did indeed dimerize, we used an assay for dimerization first described by Hope and Struhl¹⁷. The $F_B G_Z$ -core peptide and the GCN4 protein were co-translated and the resulting products were tested for TRE binding ability. In Fig. 2e, lane 8, three bands corresponding to protein/TRE complexes can be seen: the slowest migrating corresponds to the GCN4 protein bound to the TRE (compare to lane 1) and the most rapid corresponds to $F_B G_Z$ -core peptide bound to the TRE (compare to lane 4). The presence of an intermediate band is indicative of a TRE-bound heterodimer between GCN4 and $F_B G_Z$ -core (Fig. 1b). Under the same conditions, the $F_B G_Z$ -core product does not form a high affinity DNA-binding heterodimer with the Jun protein (lane 7).

We suggest that the $G_B F_Z$ -core peptide, which carries a functional GCN4 basic motif, cannot bind the TRE in Fig. 2e, lane 5, because the Fos leucine zipper does not allow the formation of homodimers. However, as $G_B F_Z$ -core can associate with Jun (Fig. 2c), we asked whether a $G_B F_Z$ -core/Jun complex is capable of binding to DNA. Figure 2e (lane 9) shows that a mixture of $G_B F_Z$ -core peptide and Jun can bind the TRE site with high affinity when compared with the separate binding capacity of $G_B F_Z$ -core peptide (lane 5) or Jun (lane 2) alone. This result indicates that the basic motifs of GCN4 and Jun can generate a functional DNA binding domain when juxtaposed by the interaction of leucine zippers from Fos and Jun (Fig. 1b).

The ability of $G_B F_Z$ -core to form a functional complex with Jun suggests that a 54-residue portion of Fos containing the leucine zipper and 24 additional C-terminal residues can confer

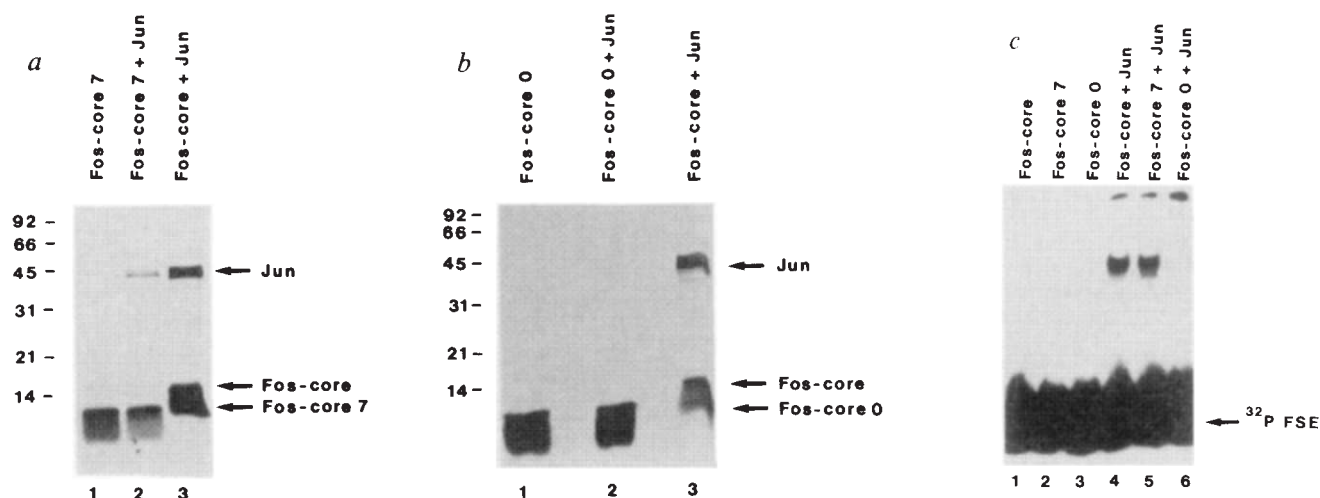


FIG. 3 Defining the limits of the Fos leucine zipper. *a*, The Fos-core 7 peptide was immunoprecipitated either prior to (lane 1) or after incubation with the Jun protein for 30 min at 37 °C (lane 2). Lane 3 shows a control precipitation of Fos-core after incubation with the Jun protein. *b*, Immunoprecipitation of the Fos-core 0 peptide either before (lane 1) or after incubation with the Jun protein for 30 min at 37 °C (lane 2). In lane 3 the Fos-core peptide was incubated with Jun and then precipitated, as a control. *c*, A mobility-shift assay in which the [³²P]-labelled FSE oligonucleotide was incubated with Fos-core, Fos-core 7 or Fos-core 0 either separately (lanes 1–3) or with the

Jun protein (lanes 4–6). The binding reaction was carried out at 37 °C for 30 min and the resulting complexes were resolved on a 4% non-denaturing polyacrylamide gel.

METHODS. *In vitro* mutagenesis of Fos-core to generate Fos-core 7 and Fos-core 0 was as described¹⁹. All the [³⁵S]-labelled proteins described were made by *in vitro* transcription and rabbit reticulocyte lysate translation as described previously⁴. An antibody generated against TrpE-Fos fusion protein (2 μl) was used for immunoprecipitation. The products were resolved by 17% SDS-PAGE. Markers on the left, $M_r \times 10^3$.

Jun binding specificity on a heterologous DNA binding domain. To define more precisely the C-terminal boundary of the sequences which confer Jun binding, we introduced stop codons at positions following the fifth leucine of the Fos zipper. Figure 3a shows that Fos-core 7, a 75-residue peptide which terminates only seven residues following the fifth leucine, is still capable of forming a complex with Jun (lane 2), and can still bind to a TRE when complexed with Jun (Fig. 3c). Deletion of seven more residues from Fos-core 7 generates Fos-core 0, which ends directly after the fifth leucine of the Fos zipper. This peptide cannot form a complex with Jun (Fig. 3b) or bind to a TRE site when in the presence of Jun (Fig. 3c).

These data, taken together with the domain-swap experiments, suggest that a 37-residue portion of the Fos and a 30-residue portion of GCN4 contain all the information required for the specific interaction of Fos with Jun and of GCN4 with itself. Because these domains essentially span just the leucine repeat region, residues that lie between these leucines must confer the specificity and affinity of zipper-zipper interaction.

Our data indicate that the basic motifs of Fos, Jun and GCN4 are comparable with respect to their TRE binding potential and that this potential can only be realized when two such basic motifs are juxtaposed within a dimeric complex. We show that the specificity (and probably affinity) of dimer formation is dictated by the adjacent leucine zipper domain and, as a consequence, this domain controls the DNA-binding ability of a given basic motif (Fig. 4). A zipper such as that of GCN4, which allows the formation of stable homodimers, generates a high-affinity DNA-binding domain. A zipper such as that of Fos, which cannot direct the formation of homodimers, fails to realize the DNA binding potential of its adjacent basic motif, but when allowed to dimerize with a compatible leucine zipper, such as that of Jun, it generates a functional, high-affinity DNA binding heterodimer (Fig. 4). In circumstances where a changing population of Fos/Jun transcription factor family members is induced in stimulated cells, these properties of the zipper and basic motif could generate a population of Fos/Jun dimers, with similar TRE binding abilities but capable of conferring distinctive transcriptional effects on TRE-regulated genes. □

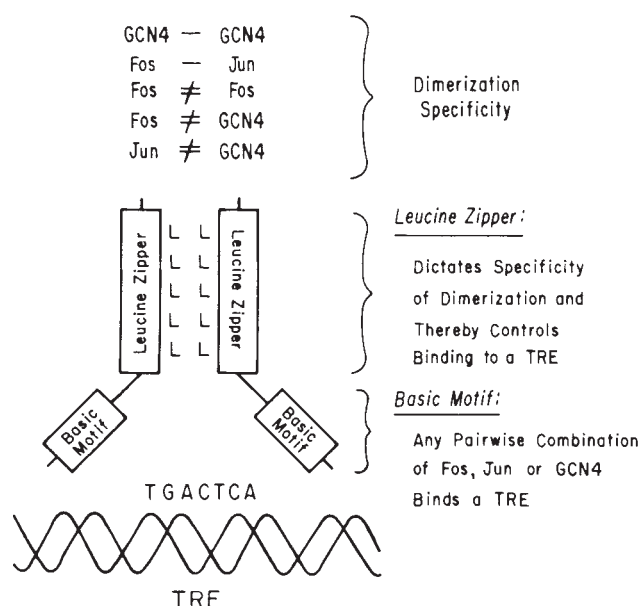


FIG. 4 Diagrammatic representation of the dimeric DNA-binding domain from the Fos/Jun family, showing the leucine zipper and the DNA binding basic motif. The type of leucine zipper present next to a basic motif dictates the specificity for another zipper domain. Above the diagram, the allowed and disallowed dimers are indicated. Dimerization results in a DNA-binding domain with high affinity for the TRE.

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Single amino-acid changes in HIV envelope affect viral tropism and receptor binding

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INFECTION by the human immunodeficiency virus (HIV) is initiated by the binding of its extracellular envelope glycoprotein, gp120, to the CD4 antigen on target cells¹⁻³. To map the residues of the HIV-1 glycoprotein that are critical for binding and to analyse the effects of binding on viral infectivity, we created 15 mutations in a region of gp120 that is important for binding to CD4 (refs 4,5). We find that substitution of a single amino acid (tryptophan at position 432) can abrogate CD4 binding and that virus carrying this mutation is non-infectious. By contrast, other amino-acid changes in the same region do not affect CD4 binding but restrict viral tropism: virions containing isoleucine substitutions at position 425 lose their ability to infect a monocyte cell line (U937 cells) but can still infect T-lymphocyte cell lines (CEM, SUP-T1) and activated human peripheral blood lymphocytes. These results indicate that cellular tropism of HIV can be influenced by a single amino-acid change in gp120.

We introduced two short deletions and 13 amino-acid substitutions (Fig. 1) in a region of the HIV envelope that is highly conserved between HIV-1 and HIV-2 (refs 6,7). Insertional mutagenesis at this site is known to abrogate CD4 binding⁴ and the epitopes for two antibodies that inhibit CD4-gp120 interaction map near this region^{5,8}. The envelope gene was expressed by transfection with a vector⁹ in which the coding sequences for the transmembrane glycoprotein gp41 are eliminated so that free gp120 is secreted into the tissue culture medium after transfection. Aliquots of supernatants from metabolically labelled transfected cells were analysed for total secreted gp120 by immunoprecipitation with an anti-gp120 monoclonal antibody. The CD4-binding activity of the mutant gp120 molecules was assayed by incubation of the metabolically labelled protein with

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excess unlabelled soluble CD4 (ref. 10), followed by immunoprecipitation of the gp120-CD4 complexes with an antibody raised against CD4 (OKT4). The intensity of the band co-migrating with gp120 is an indication of the quantity of gp120 that bound to CD4.

Neither the deletions nor the substitutions interfered grossly with the processing or maturation of the products shed into the supernatant of transfected cells (Fig. 1, and data not shown). Also, 6 out of 15 of the mutant proteins bound to CD4 in the same way as gp120 (Figs 1 and 2a), indicating that at least some of the conserved amino acids in this region are not critical for receptor binding. The deletion of several amino acids and the substitution of the tryptophan residue at position 432 by valine, glycine, serine or arginine could both eliminate CD4 binding however (Figs 1 and 2a). Although we cannot yet determine whether amino-acid substitution at Trp 432 affects the binding site directly or is causing a change in conformation elsewhere in the molecule, it may be significant that even the more conservative amino-acid substitutions of Trp 432, by phenylalanine or tyrosine for example, decrease the CD4-binding capacity of gp120 by 80% (Figs 1,2).

The isoleucine at position 425 is conserved among all strains of HIV-1, HIV-2 and simian immunodeficiency virus sequenced so far⁷, and so there must be some selective pressure to maintain this residue. However, substitution with lysine (Ile 425→Lys) reduces but does not eliminate binding, and substitution with threonine (Ile 425→Thr) has no effect (Figs 1 and 2a). Moreover, we found that the Ile 425→Thr glycoprotein and wild-type gp120 (but not Trp 432→Phe and Ile 425→Lys) have a similar pattern of competition for CD4-binding against excess non-labelled purified gp120 (Fig. 2b), indicating that the conservation of isoleucine at position 425 is not a consequence of an indispensability to gp120 for CD4 binding.

We introduced mutations into an infectious HIV-1 proviral clone: virus particles were produced by transfection of mutant proviruses in SW480 cells. These cells transiently produce large amounts of virus after transfection¹¹ but cannot be infected efficiently by HIV. We then tested cell-free supernatants from the transfected SW480 cells for ability to infect CD4⁺ cells productively by measuring reverse transcriptase activity or the amount of p25^{gag} (also called p24^{gag}) in supernatants from infected cultures at 2- or 3-day intervals. The same number of reverse transcriptase units from the supernatant of SW480 cells transfected with different plasmids was used to infect the lymphocytic cell line SUP-T1 (ref. 12) and the monocytic cell line U937 (ref. 13).

FIG. 1 Amino-acid sequence and CD4-binding activity of gp120-containing mutations in the hyperconserved region 5 (ref. 6). The numbering system is based on the sequence of gp120 from an infectious clone of HIV-1 BRU and the sequence between amino acids 423 and 433 is shown at the top. Dashes indicate that an amino acid is identical to that in the BRU prototype²⁰. Delta 6 and delta 9 are deletions prepared by site-directed mutagenesis of the amino acids shown between the brackets. Mutations are named according to the wild-type amino acid and its position followed by the amino acid to which it has been changed. METHODS. The *Pst*I-*Sac*I fragment excised from the expression vector pME240 (ref. 9) was subcloned in a M13mp18 vector. Mutagenesis was achieved by annealing degenerate oligonucleotides to a deoxyuridine-substituted single-stranded template²¹; mutations characterized by sequencing were then introduced into pME240 by inserting the *Bgl*II-*Bgl*II or *Nhe*I-*Sac*I fragments.

Wild-type virus can infect both cell lines, although infection is faster and the p25 level and reverse transcriptase activity higher in SUP-T1 compared with U937 cells (Fig. 3a and b). Virus in which Trp 432 is substituted with Phe or Ser fails to initiate productive infection in either cell line (Fig. 3a). Thus, virus infectivity correlates with the extent of binding of soluble molecules.

When virus in supernatant from SW480 cells transfected with a proviral clone containing the mutations Ile 425→Thr or Ile 425→Lys was used to infect SUP-T1 cells, the level of p25^{gag} or the reverse transcriptase activity, as well as the infection kinetics, were the same for the wild-type and the two mutants (Fig. 3a and b). Moreover, the titre of virions produced after infection was similar: in tissue culture the 50% infective dose, using positive reverse transcriptase activity as endpoint, was $10^{-4.2}$, $10^{-4.5}$ and $10^{-4.0}$ for the wild type, Ile 425→Thr and Ile 425→Lys mutants, respectively. The two mutants could also infect phytohaemagglutinin-stimulated human peripheral blood lymphocytes.

Surprisingly, neither mutant virus could productively infect U937 cells when the same criteria were used to assay virus production (Fig. 3a,b). This was the case even when U937 cells were infected at higher multiplicities of infection (m.o.i. ~1) with particles produced in SUP-T1 cells (that is, supernatant from day 9 of infection; see Fig. 3), or when viral production was monitored for more than 70 days.

We also infected U937 and SUP-T1 cells with dilutions of wild-type and Ile 425→Thr mutant virus and after 10 days plated the infected cells on a monolayer of HeLa T4 cells (HT4-6C cells; ref. 14) which had been modified by the integration of a β -galactosidase gene 3' to an HIV long terminal repeat (J. F. Nicolas, manuscript in preparation). After a further two days, cells were fixed, X-gal (a β -galactosidase substrate) was added, and the syncytia formed in the monolayer counted. This assay is more sensitive than reverse transcriptase assay at the endpoint as individual infected cells can easily be detected in the monolayer because of their bright blue colour (presumably as a result of transactivation of the β -galactosidase gene driven from the HIV long terminal repeat). We found a difference of less than fivefold in the titre of wild type compared with Ile 425→Thr mutant on SUP-T1 cells (3×10^5 versus 1×10^5 infectious units per ml) and a difference of more than 100-fold on U937 cells (5×10^3 versus 1×10^1 infectious units per ml). This shows that the Ile 425→Thr mutation quantitatively favours virus growth in one cell line over the other and so is apparently restricting viral tropism. Also, mutations of Gln 427 to Thr, and

	Amino-acid sequence	CD4 binding	Viral infectivity in	
			SUP-T1	U937
HIV1-BRU (wild type)	C R I K Q F I N M W Q E	++++	+	+
Mutants :				
Deletion 9	— [— — — — — —] — —	—	—	—
Deletion 6	— — [— — — — —] — — —	—	—	—
Ile 425-Thr (I425-T)	— — T — — — — — — — —	++++	+	—
Ile 425-Lys (I425-K)	— — K — — — — — — — —	++	+	—
Lys 426-Thr (K426-T)	— — — T — — — — — — —	++++	+	—
Lys 426-Arg (K426-R)	— — — R — — — — — — —	++++	+	+
Gln 427-Thr (Q427-T)	— — — — T — — — — — —	++++	+	—
Asn 430-Tyr (N430-Y)	— — — — — — Y — — — —	++++	—	—
Asn 430-His (N430-H)	— — — — — — H — — — —	++++	—	—
Trp 432-Tyr (W432-Y)	— — — — — — — Y — — —	++	—	—
Trp 432-Phe (W432-F)	— — — — — — — F — — —	++	—	—
Trp 432-Ser (W432-S)	— — — — — — — S — — —	—	—	—
Trp 432-Gly (W432-G)	— — — — — — — G — — —	—	—	—
Trp 432-Val (W432-V)	— — — — — — — V — — —	—	—	—
Trp 432-Arg (W432-R)	— — — — — — — R — — —	—	—	—

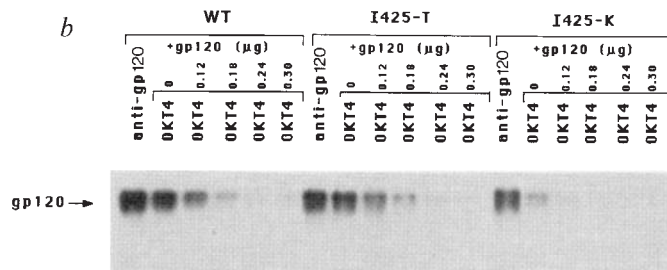
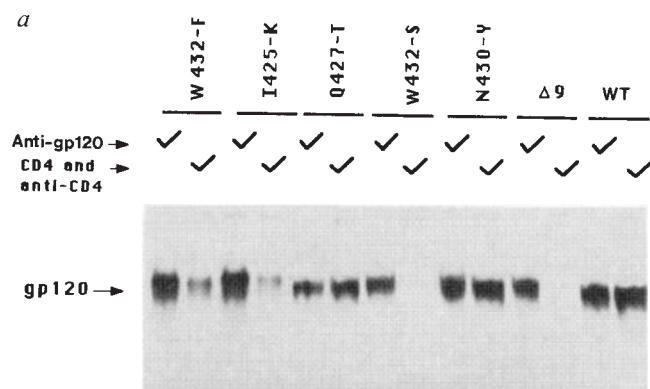


FIG. 2 Binding of mutant gp120 to CD4. *a*, Immunoprecipitation with anti-gp120 and anti-CD4 co-immunoprecipitation of gp120 from supernatants of COS-1 cells labelled with [35 S]cysteine and transfected with the gp120 constructs. COS-1 cells were transfected as described⁹. The cell-culture supernatant was used for immunoprecipitation with the monoclonal anti-gp120 antibody 110-4 (ref. 22) and for co-immunoprecipitation with the

monoclonal anti-CD4 antibody OKT4 after incubation with soluble CD4, as described in refs 3 and 6. *b*, Competition of wild-type and mutant gp120 for binding to soluble CD4. [35 S]-labelled wild-type (WT), I₄₂₅ → T or I₄₂₅ → K gp120 was incubated with soluble CD4 in the presence of increasing quantities of unlabelled, purified wild-type gp120 before immunoprecipitation of the complexes with OKT4.

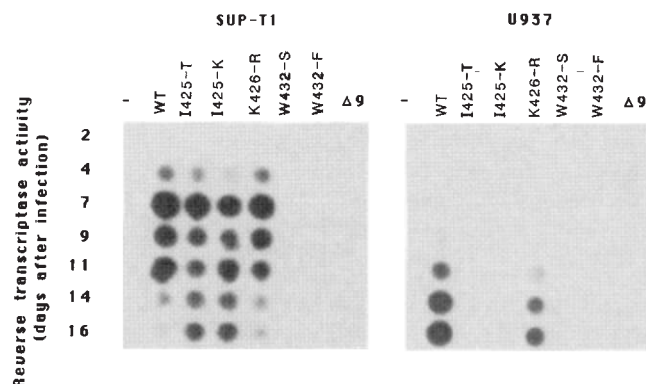
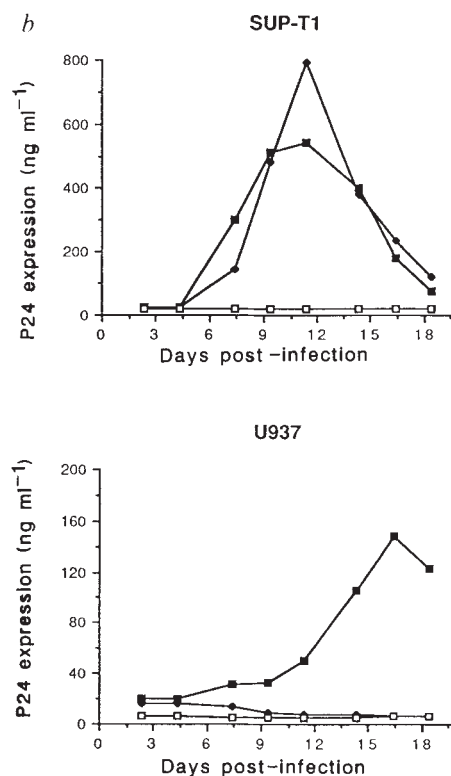


FIG. 3 Effect of *env* gene mutation on infectivity of virus particles. The proviral clone pNL4.3 (ref. 12) was first modified by substituting the fragment from *Sall*-*Bam*HI with the equivalent fragment from HIV-1 BRU. The mutant proviral clones were constructed as follows: mutant *Bgl*III-*Bgl*III fragments were first returned to a subcloned *Eco*RI-*Bam*HI fragment (5,325-8,068) from BRU isolate²⁰ and these *Eco*RI-*Bam*HI fragments containing *env* mutations were then introduced into pNL4.3. The presence of each mutation was confirmed by direct sequencing of the initial *Bgl*III fragment and of the final plasmid DNA used in individual transfections. Virus mutations are labelled as in Fig. 2. Plasmid DNA (10 μg) from the infectious molecular HIV-1 clone pNL4.3 in which the mutant *env* gene of BRU was inserted was used to transfect SW480 cells growing in 28-cm² tissue-culture flasks using a modification of the calcium phosphate precipitation method²³. Infectivity of progeny virion in the transfected-cell supernatant was determined by assaying for reverse transcriptase activity or p25/p24^{gag} expression (or both). Comparable amounts of cell-free virus were used to infect 2 × 10⁶ SUP-T1 or U937 cells and the cells were cultivated at a density of 1 × 10⁶ cells ml⁻¹ in RPMI medium containing 10% fetal calf serum and 2 μg ml⁻¹ polybrene. *a*, Aliquots of the culture fluid were assayed for reverse transcriptase activity at the times indicated by measuring [32 P]dTP incorporation using poly(A) as template and oligo(dT) as primer; reaction products were spotted on DE81 paper and exposed to film²⁴. *b*, Expression of p25/p24^{gag} in the culture fluid of cells infected with either wild-type (■), I₄₂₅-K (◆), or mock virus (□). An enzyme-linked immunosorbent assay was used to detect p25/p24^{gag} according to the manufacturer's (Elavisa Agt kit-Diagnostics Pasteur) instructions. Culture supernatants were diluted appropriately beforehand so that the assay would be in the linear response range. Experiments shown in *a* and *b* are independent of one another.



of Lys 426 to Thr have the same phenotype, growing in SUP-T1 but not in U937 cells (Fig. 1). By contrast, virus with the conservative change of Lys 426 to Arg grows in both cell lines (Fig. 3a).

CD4 acts in both SUP-T1 cells (lymphocytic) and U937 cells (monocytic) as the virus receptor^{15,16} and is comparably represented on the surface of both cells (R. Olivier, personal communication). Because Ile 425→Thr has CD4-binding activity indistinguishable in our assay from wild type (Fig. 2b), changes in isoleucine 425 are probably affecting the tropism of HIV irrespective of any effect on CD4 binding *in vitro*, unlike changes in tryptophan 432. On the other hand, the presentation of CD4 on U937 and SUP-T1 cells could differ sufficiently for small changes in envelope protein structure to affect the cells differently. The interaction of gp120 and CD4 when anchored in their respective membranes may therefore not be properly represented by our test involving soluble molecules. Second, requirements for entry of the virus after binding to its receptor may differ in different cell types, and could be affected by mutations in amino-acid residue 425. Because mutations restricting the tropism map near mutations that abolish CD4-binding, this region may be involved in transmitting conformational changes in gp120 after binding to the receptor. Third, as U937 cells are more difficult to infect than SUP-T1, even with wild-type virus (Fig. 3b), subtle changes in viral infectivity could be more critical in these cells.

Regardless of mechanism, our results indicate that single amino-acid changes in gp120 can restrict the tropism of HIV among different CD4⁺ cell lines (Ile 425) and can abolish the high-affinity binding to CD4 (Trp 432). As the wild-type clone used here is unable to infect fresh monocyte cells (data not shown), other mutations must be responsible for a strict monocyte/macrophage tropism *in vivo*. Given the enormous variability in the envelope protein during viral growth even within the same host, mutations that change the binding affinity for the receptor, or the viral tropism, could give rise to viral populations with varying pathogenicity. Indeed, a differential tropism has been demonstrated for HIV isolates which correlates with disease state¹⁷, or with the tissue from which the virus was isolated^{18,19}. The relationship between amino-acid sequence variation and virus tropism and its possible correlation with disease conditions could be significant to the understanding of HIV pathogenesis. □

Exon shuffling by recombination between self-splicing introns of bacteriophage T4

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THE organization of genes into exons separated by introns may permit rapid evolution of protein-coding sequences by exon shuffling¹. Introns could provide non-coding targets for recombination, which would then give rise to novel combinations of exons. Evidence to support this theory is indirect and consists of examples of homologous domains of protein structure encoded in different genes, with introns in conserved positions at the boundaries of these domains². Here, we report the first direct evidence for exon shuffling. Two spontaneous deletion mutations of phage T4 have been characterized by sequencing, and they are clearly the result of recombination between homologous regions of two self-splicing group I introns. As a result of the recombination, exons of different genes are transcribed together, with a hybrid intron between them. One of these introns is proficient in self-splicing.

Bacteriophage T4 has at least three closely related self-splicing group I introns in genes coding for proteins³. One of these introns is in the *td* gene, which codes for thymidylate synthase⁴, and another is in the *nrdB* gene, which codes for the small subunit of ribonucleoside diphosphate reductase^{5,6}. These two genes are near each other on the T4 genetic map, are transcribed in the same direction and have only non-essential genes between them⁷. Two sets of spontaneous-deletion mutants of T4 have been isolated which are both *td*⁻ and *nrd*⁻. One set (*far* mutants) was selected by folate analogue resistance⁸, and the other (*del63-32* mutants) were isolated by selecting for mutants that compensate for a duplication in another region of the genome⁹. Genetic mapping studies with point mutations suggest that some of these deletions extend from the 3' functional domain of the *td* intron to the corresponding region of the *nrdB* intron (our unpublished data).

The self-splicing group I introns of bacteriophage T4 have secondary structures typical of the other group I introns of mitochondria, chloroplasts and nuclear genes of eukaryotic protists³. The T4 introns also exhibit extensive primary sequence homology, some of which is also conserved among all the group I introns¹⁰. There are two long regions of similar sequence in the *td* and *nrdB* introns (Fig. 1). The sequence including P7.2 is identical in *td* and *nrdB* at 20 of 21 positions, whereas that spanning the 3' portion of P7 and all of P9 is identical at 29 of 32 positions. Because recombination within direct repeats is the most common mechanism for formation of long deletions in T4 (ref. 11), it is possible that deletion endpoints within these conserved regions could produce functional hybrid introns.

The precise boundaries of the deletions in the *farP22* and *del(63-32)1* mutants were determined by sequencing the respective RNAs (Fig. 2). In each case the recombination site was within one of these regions of extensive sequence similarity. In *del(63-32)1* RNA, the sequence (reading from 3' to 5' on the template) is identical to that of the *nrdB* intron, up until a substitution of A to G at position 1,740, indicating recombination within the nine nucleotides encompassing the 3' portion of P9. For *farP22*, the switch to the *td* sequence occurs at position 1,672 (A instead of C), indicating recombination within the 11 nucleotides encompassing the 5' portion of P7.2. In each case, the sequence 5' of the indicated nucleotide is that of the *td* intron.

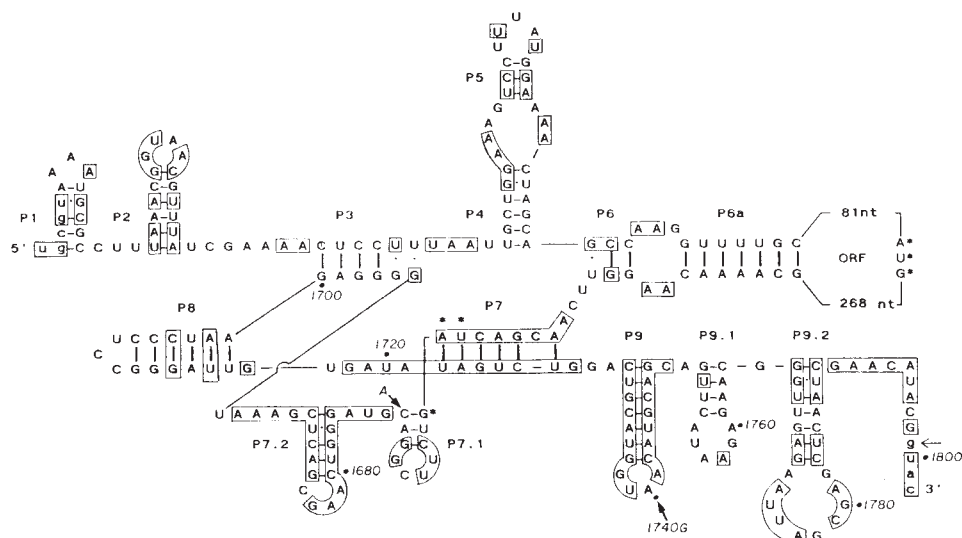
To determine if either of these hybrid introns is excised *in vivo*, we determined the sequence of RNA from infected cells

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FIG. 1 Sequence similarity between the *td* and *nrdB* introns. The *nrdB* intron is shown, with residues numbered according to ref. 5. Intron residues, upper case letters; exon residues, lower case letters, with asterisks indicating the boundaries of an open reading frame (ORF). Regions of complementary base pairing (P) are numbered according to the group I convention²⁰. The *td* intron consists of homologous structures (P1-P9.2), and also has a long ORF (of sequence unrelated to that of *nrdB*) inserted within the loop of P6a. Nucleotides that are identical in the *td* intron structure are boxed. Thick arrows delineate the boundaries of deletions that create hybrid introns: *del*(63-32)1 (A_{1,740}) and *farP22* (C_{1,672}). In each case, the 3' portion of the intron is the same as in *nrdB*; the first nucleotide that is unique to the 5' portion of the *td* intron is indicated. Thin arrow, 3' terminal G of hybrid *del*(63-32)1 intron.



using a primer complementary to the 5' portion of the second exon of *nrdB*. If splicing has occurred, a unique sequence up to the splice point should be observed, whereupon two sequences (that of precursor and ligated exons) should be superimposed. If splicing has not occurred, however, a unique sequence, identical to that of the DNA, should be obtained. The results (Fig. 3) show that, whereas *farP22* does not produce detectable quantities of spliced RNA *in vivo*, RNA from *del*(63-32)1 is extensively spliced. Interestingly, the splice point of RNA from *del*(63-32)1 is not exactly the same as that of the *nrdB* intron. Instead, the 3' end of the intron (which is always a G) occurs at position 1,799, one nucleotide 3' of the normal *nrdB* splice site³. At this point, two interdigitated sequences can be read. The second sequence is characterized by bands that have slightly faster mobilities than their counterparts resulting from precursor RNA. Although structure in the template RNA prevents an unambiguous reading of the second sequence, the data are consistent with a 5' splice site identical to that of the first *td* exon.

To determine whether this hybrid intron is capable of self-splicing, RNA from *del*(63-32)1 was incubated *in vitro* with [α -³²P]GTP under conditions that label only the 5' ends of self-excised group I introns of phage T4 (ref. 6). This labelled RNA hybridized to cloned DNA from the *td* intron on a nitrocellulose membrane (data not shown). As the sequence of the hybrid intron is almost 95% derived from *td*, these results indicate that the *del*(63-32)1 intron is excised under self-splicing conditions *in vitro*.

These results are consistent with current knowledge of the functional requirements of group I introns. Mutations that disrupt the long-range pairing element P3 are defective in splicing¹². It is therefore not surprising that the hybrid intron of *farP22*, whose 5' and 3' elements of P3 come from different introns and are not complementary, should be non-functional. On the other hand, in the case of the hybrid intron of *del*(63-32)1, all of the

structural elements known to be required for catalytic activity and 5' splice site specificity are derived from the *td* intron, and we therefore observe self-cleavage at the normal *td* 5'-splice site. The requirements for specificity of selection of the 3' splice site are not as clear. Although several features of the sequence near the 3' end of group I introns have been proposed to play a role in 3' splice-site selection^{13,14}, none of them provides a rationale for the shift of one residue (to the G adjacent to the normal 3' end of the *nrdB* intron).

The exon-shuffling hypothesis provides an explanation based on genetic selection, for the persistence of introns in modern protein-coding genes of eukaryotes¹. A recent demonstration that intron positions are conserved in homologous genes of both eukaryotic and prokaryotic origin, implies that introns are a very ancient feature of gene structure¹⁵. It has been suggested that recombination between RNA molecules may have produced diversity in an early 'RNA world'¹⁶. The results reported here represent the first verification that recombination between introns can indeed bring exons together in new combinations. Recombination at the DNA level between conserved sequences of group I (or, by analogy, group II) self-splicing introns, can therefore produce functional hybrid introns still capable of self-splicing. This has the desired feature of allowing exon shuffling importance in generating early biological diversity^{1,17-19}, while also making use of the highly efficient recombination of homologous DNA.

Recombinational events like those reported here are not rare. Recombination in phage T4, including that involved in formation of long deletions, occurs primarily within regions of DNA sequence homology¹¹. For example, of the 11 independent spontaneous deletions in the *del*(63-32) set⁹, 8 others have endpoints close to those of *del*(63-32)1 and may represent formation of hybrid introns similar to those reported here. A deletion is a simple way of bringing two nearby exons together: only a single

FIG. 2 Sequence of *td-nrdB* hybrid introns. Sequences of RNA from a, *del*(63-32)1 and b, *farP22* are compared with RNA from cells infected with wild type (wt) T4. Arrows indicate the point at which the hybrid intron sequence departs from that of *nrdB*. Letters (bottom) indicate RNA bases complementary to the dideoxynucleotides (ddNTP) present in each reaction (O indicates no ddNTP).

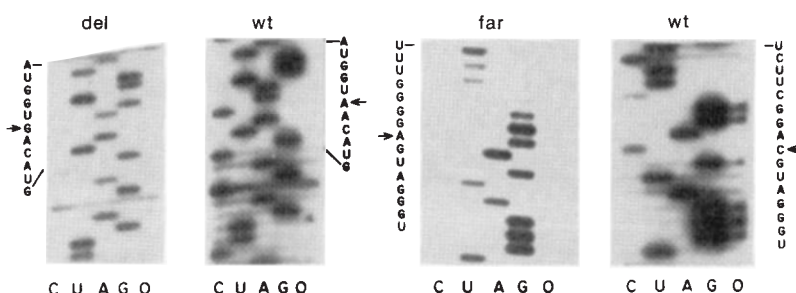
METHODS. RNA isolation at 7 min after infection of *Escherichia coli* B₆ (at 30 °C) and primer extension sequencing with reverse transcriptase were carried out as previously described³. Primers were complementary to the following portions of the *nrdB* sequence⁵:

a, 5'-ATCGAACATACGGTACCTTTAACT-3'

(coordinates 1,787-1,810);

b, 5'-GGGATTGTGATATAGTCTGG-3'

(coordinates 1,710-1,729).



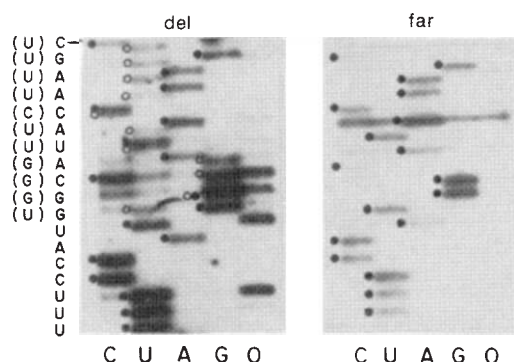


FIG. 3 Splicing of hybrid introns. Sequencing of RNAs from phages carrying deletions were primed with an oligonucleotide complementary to the 3' exon of *nrdB*. The sequence shown on the side is that of the *nrdB* precursor RNA; the sequence in parentheses is that of the 5' exon of *td*. Symbols indicate bands in positions predicted by the sequence of *nrdB* precursor (closed circles) and *td* exon 1 (open circles). Letters at the bottom are RNA bases complementary to the ddNTP present in each reaction (0 indicates no ddNTP). METHODS. RNA extraction and sequencing were as in Fig. 2, with the following changes: the primer was complementary to 5'-GCACGTGAT-GAACAGCTTCACC-3' (coordinates 1,864-1,885) in exon 2 of *nrdB*. Strong bands in the 0 lane from *del*(63-32)1, in the vicinity of the 3' splice site, probably reflect blocks to reverse transcriptase caused by stable structures in the precursor RNA. This resulted in strong bands in each sequencing reaction at these positions (data not shown). Addition of terminal transferase during the chase portion of these sequencing reactions²¹ partially removed the spurious bands.

crossover event is required. In cases where the exons are separated by essential genes or are on different chromosomes, however, two crossovers of the type described here would be required to bring exons together in a new combination.

We expect that exon shuffling can also occur in genes containing introns that are not of the self-splicing type, but with a lower frequency as there are fewer and less extensive regions of homology. It will be interesting to see if the many deletion mutants available in other organisms, including those in organelles containing self-splicing introns, provide additional examples of exon shuffling similar to the ones described here. □

Note added in proof: We have recently described an interaction, between nucleotides in the intron core (between P7 and P9) and the penultimate intron nucleotides, which is a determinant of the 3' splice site (F. Michel, *et al.*, submitted). In the hybrid intron from *del*(63-32)1, this interaction predicts the shift of one residue at the 3' splice site that is seen in Fig. 3.

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Physical mapping of complex genomes

D. Garza, J.W. Ajioka, J.P. Carulli, R.W. Jones, D.H. Johnson and D.L. Hartl

The polymerase chain reaction can be used to create probes for specific regions of the *Drosophila* genome and for chromosome walking in a library of yeast artificial chromosomes.

Two extensions of the polymerase chain reaction (PCR)¹ have been developed to facilitate the large-scale physical mapping of the genome of *Drosophila melanogaster*. In the first — termed 'microdissection PCR' — PCR is used for the enzymatic amplification of DNA fragments obtained by the dissection of particular regions of salivary gland chromosomes². Probes created in this manner can be used for the initial screening of a *Drosophila* library of yeast artificial chromosomes (YACs)³. The second technique, 'inverse PCR', involves circularizing DNA fragments so that a region of unknown sequence becomes flanked by regions of known sequence, and so can be amplified⁴. Probes obtained from inverse PCR can be used to verify putative overlaps between YAC clones and for chromosome walking within the YAC library⁵⁻⁷. We are now using these methods to establish a linked set of YACs spanning approximately 1.2 million base pairs in the 83C-84B region of the *Drosophila* genome.

The euchromatic portion of the *Drosophila* genome consists of approximately 100,000 kilobases⁸ of DNA, laterally replicated about 1,000-fold in the giant salivary gland chromosomes to produce roughly 5,000 transverse bands⁹. A typical YAC clone contains sufficient *Drosophila* DNA to allow *in situ* hybridization across several bands. Putative overlaps between different YACs can often be visualized cytologically. We are now attempting to construct a global physical map of the *Drosophila* genome through the *in situ* hybridization of YAC clones chosen randomly from libraries prepared from the Oregon R strain that have an average insert size greater than 150 kilobases.

In many cases it is advantageous to target mapping efforts to a specific region of a genome, particularly when the physical map of a region approaches saturation, putative overlaps must be verified, and gaps need to be filled. Bacteriophage or cosmid clones can provide suitable probes for some applications¹⁰, but a more general strategy based on microdissection PCR offers advantages in that it does not depend upon preexisting clones.

In microdissection PCR, probe DNA corresponding to a specific salivary region is obtained by microdissection of salivary chromosomes, and the DNA is amplified using PCR. The region we chose for initial study was at the base of the right arm of the third *Drosophila* chromosome (salivary chromosome region 83C-84B). This region

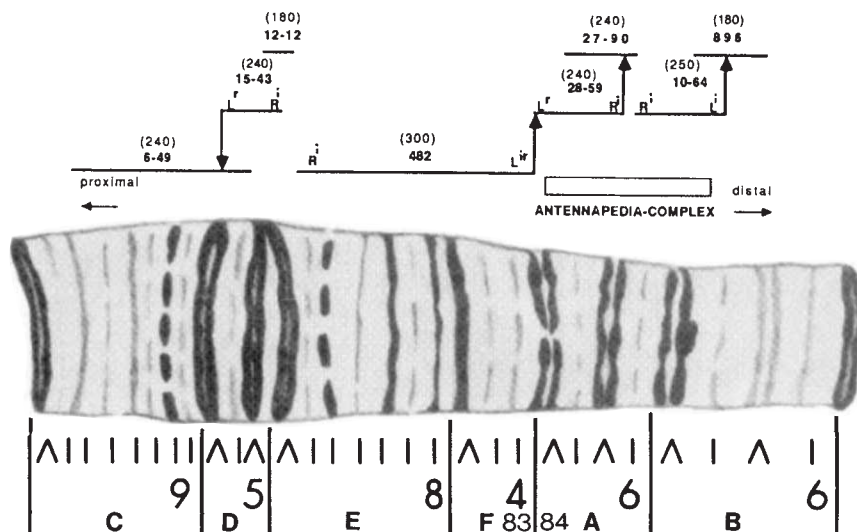


FIG. 1 Cytological and physical map of the 83C-84B region of the *Drosophila* genome. The extent of each YAC insert is shown relative to the salivary map. Numbers in parentheses are sizes of the inserts in kilobases. L and R indicate the left and right (centromere and telomere) junctions of the YAC insert compared to the salivary map. Superscripts i and r denote probes for the left or right vector-insert junctions produced using inverse PCR (i) or plasmid-rescue (r). Arrows denote clones whose overlaps have been demonstrated using the end-specific probes. Clone 27-90 was initially identified by colony hybridization using an inverse PCR probe from the right junction of clone 28-59. The open rectangle denotes a region where extensive chromosome walks have been carried out using λ and cosmid clones¹¹.

includes a number of important developmental genes, such as the homeotic Antennapedia complex¹¹ and the *Tpl* dosage-sensitive region¹².

Briefly, squashed salivary chromosomes are microdissected, extracted, and digested to completion with *Mbo*I, and adapter DNA sequences are ligated onto the ends of the *Mbo*I fragments. The adapters are formed by annealing a 5'-phosphorylated, 24-base oligonucleotide to a nonphosphorylated 20-mer, to produce a 20-mer with an *Mbo*I sticky end. Using the 20-mer as a primer, the microdissected DNA fragments can be amplified with 35-40 cycles of PCR to yield approximately 100 ng of DNA².

We amplified DNA from the entire 83C-84B region and from two sequential microdissections across the region (83C-83E and 83F-84B) and used it to probe filters bound with a total of 3,466 yeast colonies containing YACs of *Drosophila* DNA. Twenty-four clones giving positive hybridization signals were chosen and 12 of these were confirmed by Southern blots with probes created using microdissection PCR. *In situ* hybridizations with salivary gland chromosomes were initially carried out using ten of the largest YACs identified. Seven of the clones hybridized primarily to the 83-84 region, one hybridized

to two euchromatic sites (one of which was in the 83-84 region), one hybridized to the chromocenter and multiple euchromatic sites, and one gave no hybridization. Five of these clones identified using microdissection PCR probes and two others previously assigned to the 83-84 region by *in situ* mapping of random clones were selected for further analysis because they appeared to span most of the 83-84 region. Both of the clones identified through *in situ* mapping also hybridized to the microdissection PCR probes.

We used inverse PCR to create probes corresponding to the junctions between the YAC vector arms and the inserted *Drosophila* DNA to identify overlaps between the YAC clones. In this procedure, YAC DNA isolated from agarose is digested with either *Eco*RV and *Hinc*II alone, or with both enzymes together. These enzymes cleave both vector arms near the cloning site, and are expected to recognize multiple restriction sites in each insert. Circularization of the resulting fragments under dilute ligation conditions yields two types of junction fragment: one containing vector sequences extending from the cloning site towards the YAC centromere; and the other containing vector sequences extending from the cloning site towards the YAC telomere.

The *Drosophila* DNA in the junction fragments can then be amplified specifically with PCR using primers homologous to the vector sequences situated with their 3' ends flanking the *Drosophila* insert in the circularized molecules¹³. The length of *Drosophila* DNA in the circularized junction fragments is determined by the position of the closest *EcoRV* or *HincII* site. We determined the expected size of the probes resulting from inverse PCR from Southern blots of yeast genomic DNA using the same restriction enzymes used to generate the junction fragments. Probes specific to the junction nearest the centromere can also be obtained by plasmid rescue³.

Probes derived from the junctions of *Drosophila* DNA inserted into YACs have several uses in mapping. *In situ* hybridization allows the inserts to be oriented within the *Drosophila* genome, and their end points to be determined cytologically. (It also reveals which probes contain sequences that are unique in the genome.) Probing Southern blots allows overlaps between YAC inserts to be identified at the molecular level, and probing the entire YAC library allows chromosome walking by identifying additional overlapping clones.

We have identified and linked sets of clones derived from the 83C-84B region of the salivary gland map (Fig. 1). Additional YACs are being sought to fill in the gaps. Restriction maps of the YACs are presently being determined to establish colinearity with λ clones from the region and to confirm the overlaps. □

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New tech in biotech

New biotechnology products will be exhibited at next week's Congress for Developmental Biologists in Utrecht, the Netherlands.

THE Koch-Light division of New Brunswick Scientific Ltd, exhibiting on stands 27 and 28, has a *Taq* polymerase enzyme for **DNA sequencing** by the dideoxy method (*Reader Service No. 101*). Koch-Light's *Taq*-Seq enzyme is a thermostable DNA polymerase that retains high activity even when DNA sequencing is performed at high temperatures. The company also offers *Taq*-Syn, a grade of *Taq* polymerase suitable for standard DNA polymerase reactions. Both *Taq*-Seq and *Taq*-Syn are £50 (UK) for 100 units.

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